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ORIGINAL ARTICLE

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An integrated experimental and physiologically based pharmacokinetic modeling study of penicillin G in heavy sows

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Abstract

Penicillin G is widely used in food-producing animals at extralabel doses and is one of the most frequently identified violative drug residues in animal-derived food products. In this study, the plasma pharmacokinetics and tissue residue depletion of penicillin G in heavy sows after repeated intramuscular administrations at label (6.5 mg/kg) and 5 × label (32.5 mg/kg) doses were determined. Plasma, urine, and environmental samples were tested as potential antemortem markers for penicillin G residues. The collected new data and other available data from the literature were used to develop a population physiologically based pharmacokinetic (PBPK) model for penicillin G in heavy sows. The results showed that antemortem testing of urine provided potential correlation with tissue residue levels. Based on the United States Department of Agriculture Food Safety and Inspection Service action limit of 25 ng/g, the model estimated a withdrawal interval of 38 days for penicillin G in heavy sows after 3 repeated intramuscular injections at 5 × label dose. This study improves our understanding of penicillin G pharmacokinetics and tissue residue depletion in heavy sows and provides a tool to predict proper withdrawal intervals after extralabel use of penicillin G in heavy sows, thereby helping safety assessment of sow-derived meat products.

KEYWORDS

extralabel withdrawal interval, food safety, heavy sow, physiologically based pharmacokinetic (PBPK) model, procaine penicillin G (PPG), tissue residue

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1 | INTRODUCTION

Penicillin G is one of the most widely used antimicrobials in food-producing animals, including swine, cattle, and sheep (FDA, 2013; Portis, Lindeman, Johansen, & Stoltman, 2012; Vogel, Nicolet, Martig, Tschudi, & Meylan, 2001). Penicillin is also among the top three most common violative residues detected in food-producing animals in the National Residue Program reports by US Department of Agriculture (USDA) from 2011 to 2016 (USDA, 2015,2017a,2017b). Animal-derived products with drug residues above the regulatory maximum residue level (MRL) or tolerance (termed violative residues) represent a global food safety concern (Baynes et al., 2016; Baynes & Riviere, 2014). Penicillin residues are of particular concern due to the hypersensitivity in some individuals. Around 7% to 10% of the general human population is allergic to penicillin and related drugs (Dayan, 1993). Available evidence has shown that consumption of beef or pork products containing violative penicillin residues can lead to anaphylactic reactions (Dayan, 1993; Gomes & Demoly, 2005; Raison-Peyron, Messaad, Bousquet, & Demoly, 2001).

In the US, intramuscular (IM) administration of procaine penicillin G (PPG) is approved at a daily dose of 6,600 IU/kg of body weight (6.5 mg/kg) for no more than seven consecutive days (Papich et al., 1993). The term extralabel (or off-label) refers to legal use a drug in an animal under the supervision of a veterinarian in the manner that is not in accordance with the approved product's label (FDA, 2017). The Animal Medicinal Drug Use and Clarification Act (AMDUCA) provides a mechanism for veterinarians to use PPG at higher doses and for additional target organisms beyond the original label. Typically used clinical doses for IM treatment are approximately 3.5–10 times the US label dose (Payne, Craigmill, Riviere, & Webb, 2006). The extralabel use of PPG can lead to violative residues if the animals are slaughtered at the time indicated on the label. To ensure animal-derived food safety, the US Food and Drug Administration (FDA) has established a zero tolerance, which is operationally equivalent to the limit of detection (LOD), in edible tissues of swine (FDA, 2013). In the US, the Food Safety and Inspection Service (FSIS) has established an action limit of 25 ng/g for penicillin residues detected in swine tissues (FSIS, 2013).

A previous study (Korsrud et al., 1998) found penicillin residues in kidneys persisted beyond the sampling timeframe of 7 days after IM administration of 5 × the label dose in market pigs. This study reported that an extended withdrawal interval (WDI) of 15 days would be required for the tissue concentrations to fall below the safety level. Very limited research is available for the depletion of penicillin in heavy sows, and they are not sufficient to establish the appropriate WDI to ensure that extralabel doses do not result in violative residue in cull sow products (Apley, Coetzee, Gehring, & Karriker, 2009). The study carried out by Apley et al. used a short depletion sampling time duration of 8 days, which was not long enough for all samples to fall below the FSIS action limit (FAL) of 25 ng/g (Apley et al., 2009). A more recent study used a longer depletion sampling time duration of 38 days and reported that an extended withdrawal interval of 51 days was needed for kidney levels of penicillin G to deplete below 25 ng/g after 5 × label dose administration (Lupton, Shelver, Newman, Larsen, & Smith, 2014). However, large variabilities among samples made the withdrawal interval estimation violate the statistical assumption of equal variances. Existing experimental data are not sufficient to predict WDIs of penicillin G using statistical analysis. Recently, a nonlinear mixed-effect (NLME) population pharmacokinetic model (Li et al., 2014) and a population physiologically based pharmacokinetic (PBPK) model (Li, Gehring, Riviere, & Lin, 2017,2018) for penicillin G in market-age swine and cattle were developed, but these models are not available for cull sows. Therefore, more residue depletion studies of penicillin G and the establishment of a population PBPK model are needed to estimate WDIs after extralabel use of penicillin G in heavy sows.

The objectives of this study were to (a) determine the tissue depletion profile of penicillin G in sows after IM administration of label dose 6,600 IU/kg (6.5 mg/kg) and 5 × label dose 33,000 IU/kg (32.5 mg/kg) once daily for 3 consecutive days using a sensitive liquid chromatography–tandem mass spectrometry (LC-MS/MS) analytical method, (b) compare the sensitivity and specificity of different analytical methods for penicillin G, (c) conduct a correlation analysis of tissue concentrations with environmental, plasma, and urine samples, and (d) develop a population PBPK model based on pharmacokinetic data from the current and other available studies and then use the model to estimate WDIs after extralabel use of PPG in sows.

2 | MATERIALS AND METHODS

2.1 | Animals

The animal protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Iowa State University before the initiation of the study. Forty-seven healthy cull sows were obtained from a commercial sow herd. None of the cull sows had previous PPG treatment for the 52 days prior to the start of the study. Sows were placed in study pens according to their allotment upon arrival and were acclimatized in their assigned pens for 72 hr. Each sow was identified by the use of a plastic livestock ear tag placed in the left ear of the sow. Three one-inch diameter, circular tattoos were applied on the skin: one each on the right and left postauricular areas and on the right hip. The entry weights were used to randomly allocate the sows into housing group based on anticipated necropsy date and treatment option. Sows were housed in 4 rooms according to their scheduled necropsy time. Housing conditions were in accordance with the recommendations in the Guide for the Care and Use of Agricultural Animals in Agricultural Use and Research and Teaching 3rd Edition. Sows were fed with an age-appropriate diet ad libitum that meets or exceeds NRC nutrient requirements and had free access to water. The feed was an age-appropriate non-mediated Nature's Match Land O' Lakes ration.

TABLE 1 Summary of pharmacokinetic studies for penicillin G in heavy sows used for calibration and evaluation of the PBPK model

PK studies	Use	Routes	*N*	BW (kg)	Tissues	Methods	Dose regimen	Dose per admin (mg/kg)	LOQ, LOD (ng/g)
Apley et al. (2009)	Calibration	IM	5	209.7	M, L, K, F	LC-MS/MS	Single injection	32.5	kidney: 50, 30; liver: 50, 19; muscle: 10, 2.4; plasma: 10, 0.6; fat: 50, 19
Lupton et al. (2014)	Calibration	IM	18	228.3	P, M, K	LC-MS/MS	24-hr interval 3 doses	32.5	kidney: 6.1, 1.8; muscle: 2.4, 0.7; plasma: 5, 1.5; urine: 4.1, 1.1
Current study	Evaluation	IM	4	238.2	P, M, L, K	LC-MS/MS	24-hr interval 3 doses	6.5, 32.5	kidney: 15, 0.2; liver: 30, 0.2; muscle: 5, 0.2; plasma: 5, 0.5; urine: 30, 5

Note. The abbreviations for routes: IM: intramuscular injection. The abbreviations for tissues: F: fat; K: kidney; L: liver; M: muscle; P: plasma. The abbreviations for methods: LC-MS/MS: liquid chromatography–tandem mass spectrometry. The abbreviations for "LOQ and LOD (ng/g)": LOD: limit of detection; LOQ: limit of quantitation.

2.2 | Treatments for the residue depletion experiments

Fifteen sows were allocated to treatment group 1 (TG1); 16 sows were allocated to TG2; and 16 sows were allocated to TG3. All sows in TG1 received 6,600 IU/kg PPG (Agricillin, 300,000 IU PPG/mL, AgriLabs, St. Joseph, MO); sows in TG2 received a sterile saline volume equivalent of 19,800 IU/kg PPG (an average of TG1 and TG3); and sows in TG3 received 33,000 IU/kg PPG. The heaviest one-third (16), middle one-third (16), and lightest one-third (15) pigs were blocked by weight. They were given a random number and assigned to a treatment group (TG1-TG3), necropsy group (G1-G4), and pen number demonstrated in Table S1. Each sow was restrained with a hog snare and individual injections were administered at a specified time for each individual sow. The injections were administered IM with a 16 gauge, 1-inch needle inside the circular tattoo. Up to 10 ml for TG1 and 20 ml for TG2 and TG3 was administered in the dorsal site and the remaining volume was given at the ventral site. Injections were administered at the same time for three consecutive days. Day 0 injections were given in the left postauricular area; Day 1 injections were given on the right postauricular area; and Day 2 injections were given on the right hip area.

2.3 | Sample collection and processing

Blood samples were obtained immediately prior to the first injection, two days after completion of dosing regimen, and immediately prior to euthanasia (two time points total for G1 and three time points total for G2-G4). Necropsies were performed on each sow assigned to the necropsy group at the assigned days postadministration. These tissue samples were stored in Whirl-Pak bags and placed on ice until permanent storage at −80°C. Environmental sampling was performed in each group to assess the presence of penicillin G in the environment. Environmental samples were collected using unscented Swiffer pads during the acclimation period before PPG administration, on Day 2 after PPG administration, and each day of necropsy. Urine samples were collected at necropsy. All samples were stored at −80°C until analysis. Detailed information about sample collection is provided in Supporting Information.

2.4 | Sample extraction for LC-MS/MS analysis

Plasma, urine, and tissue samples, including liver, kidney, muscle, and injection site, were extracted for LC-MS/MS analysis. Calibration standards were prepared by adding standard penicillin G to blank samples. Blank sample refers to samples with no known exposure to penicillin G. Internal standard, penicillin G-d7 ethylperidinium salt (Sigma, St. Louis, MO), was added to the standards/samples. Acetonitrile was added to standards/samples, followed by mixing with a vortex mixer, and then centrifugation. Supernatant was transferred to a test tube and evaporated to dryness using a stream of nitrogen. Resuspended samples were transferred to an

autosampler vial with glass insert for LC-MS/MS analysis. Please refer to Supporting Information for more detailed information.

2.5 | Sample analysis

All drug concentrations in collected samples were analyzed at Iowa State University Veterinary Diagnostic Lab and the Iowa State University-Pharmacology Analytical Support Team (ISU-PhAST). Concentrations of penicillin G in liver, kidney, muscle, injection site, plasma, and urine samples were measured using LC-MS/MS. Separation was achieved via high-performance liquid chromatography (HPLC) using a Surveyor pump and autosampler from Thermo Scientific (San Jose, CA, USA). Data collection was achieved using a Thermo TSQ Quantum Discover Max triple quadrupole mass spectrometer. The HPLC system utilized a Kinetex C18 column (100mm x 2.1 mm, 2.6 µm particle size) from Phenomenex (Torrance, CA, USA) maintained at 40°C. The mobile phase consisted of A: 0.1% (v/v) formic acid in water and B: 0.1% (v/v) formic acid in acetonitrile. The flow rate was 0.25 ml/min. The mobile phase began at 20% B with a linear gradient to 95% B which was maintained for 2 min before re-equilibration to 20% B. Both penicillin G and penicillin G d-7 had a retention time of 4.6 min. The transitions used for penicillin G identification were (m/z) 335 → 114/160/176. The transitions used for the internal standard, penicillin G-d7, were 342 → 114/160/183. All data were collected in positive ion mode. All standard curves for tissues, plasma, and urine had a coefficient of determination that exceeded 0.98. QC samples were deemed to have passed when calculated concentration values were within 20% accuracy of expected levels. The Kidney Inhibition Swab (KIS), Charm MRL, and SNAP beta-lactam tests were performed according to manufactures' instructions. More detailed methods are available in Supporting Information.

2.6 | PBPK modeling for penicillin G in heavy sows

The PBPK model for penicillin G in heavy sows was primarily based on a recently published PBPK model for penicillin G in market-age swine (Li, Gehring, Riviere, & Lin, 2017) using available values of physiological parameters for sows, and further calibrated with available pharmacokinetic data in heavy sows (Apley et al., 2009; Lupton et al., 2014). The experimental data from the current study were used to evaluate the model. The summary of these data sets is shown in Table 1. The PBPK model has seven compartments standing for different tissues connected by the circulating blood system (Figure 1). The flow-limited model was applied for all tissue compartments in the current model based on the published model structure for penicillin G in beef cattle, market-age swine, and dairy cows (Li et al., 2017; Li, Gehring, Riviere, & Lin, 2018). Berkeley Madonna (Version 8.3.23.0; University of California at Berkeley, CA, USA) was used to develop the PBPK models. Additional information for the PBPK model development can be found in our previous publications (Li et al., 2017, 2018; Lin, Li, Gehring, & Riviere, 2015; Lin, Vahl, & Riviere, 2016). The model codes are provided in the Supporting Information and will also be deposited on our website (http://iccm.k-state.edu/).

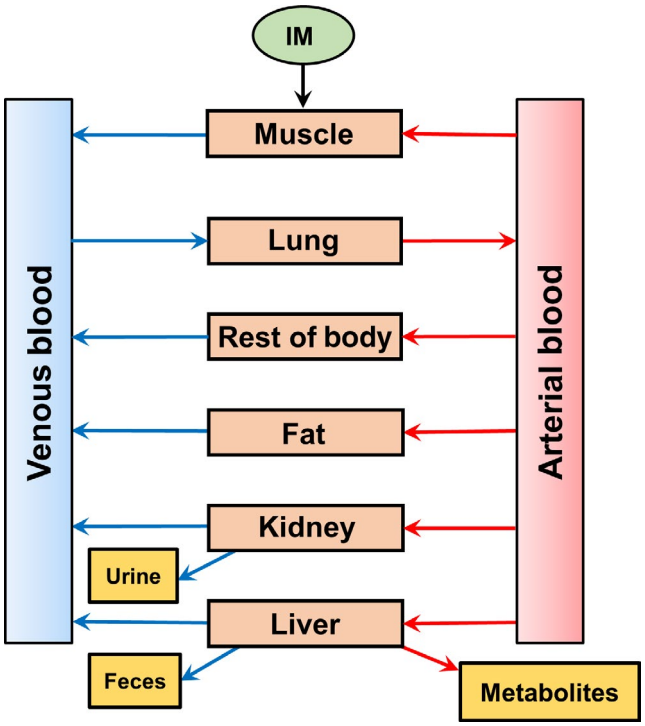

FIGURE 1 A schematic diagram of the physiologically based pharmacokinetic (PBPK) model for penicillin G in heavy sows. The label administration route of procaine penicillin G, intramuscular (IM) injections, is presented in the model [Colour figure can be viewed at wileyonlinelibrary.com]

2.7 | Model calibration and parameterization

The PBPK models have two different types of parameters including physiological parameters and chemical-specific parameters. The average values and coefficients of variance of body weight (BW), tissue volume fractions of liver (VLC), and kidney (VKC) were calculated based on experimental data from the current and other studies for heavy sows (Apley et al., 2009; Fugate, 1991). All the other physiological parameters of sows were kept the same as corresponding values for market-age swine. As for chemical-specific parameters (e.g., partition coefficients), the original values were from the previous PBPK model of penicillin (Li et al., 2017). They were optimized using the Curve Fitting module in Berkeley Madonna, and further optimized as needed by visually fitting model simulations to the calibration data sets. Values of all physiological parameters and chemical-specific parameters used in the PBPK model are provided in Table 2.

2.8 | Model evaluation

The performance of the PBPK model was evaluated by comparing model simulations with concentrations of penicillin G from the current study. According to World Health Organization guidelines (WHO, 2010), if the simulations matched the measured kinetic profiles well and were generally within a twofold range of the measured values, the model was considered reasonable and

TABLE 2 Values and distributions of parameters used in the population analysis for the PBPK model of penicillin G in heavy sows

Parameter	Abbreviation	Distribution	Mean	*SD*	CV	Lower bound	Upper bound	References
Body weight (kg)	BW	Normal	223.062	3.815E+01	0.171†	148.293	297.832	Apley et al. (2009), Fugate (1991) and current study
Cardiac output (L/h/kg)	QCC	Normal	8.543	1.910E+00	0.224†	4.800	12.287	Li et al. (2017)
Tissue volume (fraction of body weight, unitless)								
Arterial blood	VartC	Normal	0.016	4.680E-03	0.300	0.006	0.025	Li et al. (2017)
Venous blood	VvenC	Normal	0.044	1.332E-02	0.300	0.018	0.071	Li et al. (2017)
Liver	VLC	Normal	0.011	4.039E-03	0.365†	0.003	0.019	Fugate (1991)
Kidney	VKC	Normal	0.002	8.078E-04	0.333†	0.001	0.004	Fugate (1991)
Muscle	VMC	Normal	0.355	2.494E-03	0.007†	0.351	0.360	Li et al. (2017)
Fat	VFC	Normal	0.235	1.802E-02	0.077†	0.200	0.270	Li et al. (2017)
Lung	VLuC	Normal	0.010	3.000E-03	0.300	0.004	0.016	Li et al. (2017)
Rest of body	VrestC	Normal	0.326	9.778E-02	0.300	0.134	0.518	Total adds to 1
Blood flow (fraction of cardiac output, unitless)								
Liver	QLC	Normal	0.273	8.175E−02	0.300	0.112	0.433	Li et al. (2017)
Kidney	QKC	Normal	0.116	1.733E−02	0.149†	0.082	0.150	Li et al. (2017)
Muscle	QMC	Normal	0.293	4.216E−02	0.144†	0.211	0.376	Li et al. (2017)
Fat	QFC	Normal	0.128	3.825E−02	0.300	0.053	0.202	Li et al. (2017)
Rest of body	QrestC	Normal	0.190	5.712E−02	0.300	0.078	0.302	Total adds to 1
Absption rate constant (/h)								
Intramuscular	Kim	Lognormal	0.015	6.000E−03	0.400	0.007	0.030	Model fitted
	Frac	Lognormal	0.010	1.000E−03	0.100	0.008	0.012	Li et al. (2017)
	Kdiss	Lognormal	0.001	4.000E−04	0.400	0.0004	0.002	Model fitted
Tissue:plasma partition coefficient for the parent drug (unitless)								
Liver	PL	Lognormal	0.200	8.000E−02	0.400	0.087	0.395	Model fitted
Kidney	PK	Lognormal	10.000	4.000E + 00	0.400	4.364	19.756	Model fitted
Muscle	PM	Lognormal	0.300	1.200E−01	0.400	0.131	0.593	Model fitted
Fat	PF	Lognormal	0.100	4.000E−02	0.400	0.044	0.198	Model fitted
Lung	PLu	Lognormal	0.180	7.200E−02	0.400	0.079	0.356	Li et al. (2017)
Rest of body	Prest	Lognormal	0.479	1.916E−01	0.400	0.209	0.946	Li et al. (2017)
Hepatic metabolic rate (/h/kg)	KmC	Lognormal	0.050	2.000E−02	0.400	0.022	0.099	Model fitted
Percentage of plasma protein binding (unitless)	PB	Lognormal	0.366	1.464E−01	0.400	0.160	0.723	Li et al. (2017)
Urinary elimination rate constant (L/h/kg)	KurineC	Lognormal	0.800	3.200E−01	0.400	0.349	1.580	Model fitted

Note. The parameters, which were estimated by fitting the PBPK model with the available pharmacokinetic data, were marked as "model fitted." A "†" sign indicates the CV was determined based on previous experimental data.

validated. The sensitivity and linear regression analyses were carried out using the method reported previously (Cheng, Riviere, Monteiro-Riviere, & Lin, 2018; Elwell-Cuddy, Li, KuKanich, & Lin, 2018; Lin et al., 2017; Zeng et al., 2017). The linear regression analysis was based on the mean value at each time point. The uncertainty of highly sensitive parameters was designated qualitatively as low, medium, and high based on the criteria reported in Teeguarden et al. (2005). The penicillin G depletion in urine was simulated using the PBPK model. The urine production rates used in the calculation of urine concentrations were summarized in Table S2 (Chastain, Camberato, Albrecht, & Adams, 1999; Deding, Pedersen, Bjarkam, & Djurhuus, 2006; Hamilton, Luce, & Heald, 1997; Hannon, Bossone, & Wade, 1990; Patience, Friend, Hartin, & Wolynetz, 1987). Due to lack of experimental data, renal clearance of penicillin G was simulated using the first-order kinetics, and only the reported range of urine volumes was involved to simulate the penicillin G concentration in urine.

2.9 | Population PBPK model

Based on the current PBPK model, Monte Carlo simulation was applied to estimate the effects of parameter uncertainty and between-animals variability of heavy sows on model simulations. One-thousand iterations were carried out for each Monte Carlo analysis. Hypothetical populations of heavy sows with all physiological and chemical-specific parameters distributed randomly around the mean values and within the 95% confidence intervals were specified in Table 2. According to the available studies, different therapeutic scenarios were simulated using Monte Carlo analysis for heavy sows. The label dose 6,600 IU/kg of body weight (6.5 mg/kg) was simulated for single IM injection or 3 daily IM injections, and the 5 × label dose 33,000 IU/kg of body weight (32.5 mg/kg) was also simulated for 3 daily IM injections. The median, 1st, and 99th percentiles of simulated results were calculated and plotted without confidence intervals.

2.10 | Determination of Extended Withdrawal Intervals after Extralabel Use of PPG in Heavy Sows by Using the Population PBPK Model

The WDIs after label or extralabel use of PPG in heavy sows were determined using results of the Monte Carlo simulation. The FSIS has established an action limit of 25 ng/g for penicillin residues detected in swine tissues (FSIS, 2013). As there is zero-tolerance limit for penicillin G in edible tissues of swine in United States (Brynes, 2005), the WDIs can be determined as the time when 99th percentiles of the target tissue concentrations of penicillin G fall below FAL or LOD.

TABLE 3 Concentrations of penicillin G (ng/g) in kidney, liver, semitendinosus/semimembranosus muscle, and injection site(s)

		Treatment group 1						Treatment group 2		
						Injection site				
Necropsy group	Pen number	Sow ID	Kidney	Liver	Muscle	(Dorsal)	(Ventral)	Sow ID	Kidney	Liver
G1 (day 1)	1	N/A	N/A	N/A	N/A	N/A	N/A	468	<LOQ	<LOQ
	2	342	<LOQ	<LOQ	13.7	535872	N/A	463	<LOQ	<LOQ
	3	473	31.7	<LOQ	15.5	351282	N/A	462	<LOQ	<LOQ
	4	339	16.4	<LOQ	15.6	1151	N/A	471	<LOQ	<LOQ
G2 (day 6)	1	470	<LOQ	<LOQ	<LOQ	105.5	N/A	456	<LOQ	<LOQ
	2	446	96.4	<LOQ	<LOQ	895.2	652.2	466	<LOQ	<LOQ
	3	345	<LOQ	<LOQ	<LOQ	<LOQ	N/A	452	<LOQ	<LOQ
	4	474	<LOQ	<LOQ	<LOQ	97.8	N/A	350	<LOQ	<LOQ
G3 (day 14)	1	472	<LOQ	<LOQ	<LOQ	105.1	N/A	458	<LOQ	<LOQ
	2	349	<LOQ	<LOQ	<LOQ	<LOQ	N/A	347	<LOQ	<LOQ
	3	344	<LOQ	<LOQ	<LOQ	<LOQ	N/A	451	<LOQ	<LOQ
	4	440	<LOQ	<LOQ	<LOQ	<LOQ	N/A	445	<LOQ	<LOQ
G4 (day 28)	1	340	<LOQ	<LOQ	<LOQ	<LOQ	N/A	459	<LOQ	<LOQ
	2	348	48.4	<LOQ	<LOQ	207.7	N/A	460	<LOQ	<LOQ
	3	448	<LOQ	<LOQ	<LOQ	<LOQ	N/A	343	<LOQ	<LOQ
	4	465	<LOQ	<LOQ	<LOQ	<LOQ	N/A	469	<LOQ	<LOQ

Note. N/A: not available.

Concentrations that were below the level of quantification (LOQ) was designated "<LOQ." The following LOQ values were applied: kidney, 15 ng/g; liver, 30 ng/g; muscle 5 ng/g; injection site, 50 ng/g. Injections were given in two locations on the hip: "dorsal" and "ventral." For TG1, up to 10 ml of procaine penicillin G was injected in the dorsal location first, with remaining volume injected on the ventral location. For TG2 and TG3, up to 20 ml was injected in the dorsal location and remaining volume was injected ventrally.

3 | RESULTS

3.1 | Residue depletion study

Residues in TG1 sows administered the label dose of PPG were detected in kidney, muscle, and injection sites by LC-MS/MS. Kidney residues were detected in 67% of sows at Day 1 postadministration, 25% at Day 6, and in 25% at Day 28 with a level of 48.4 ng/g. Penicillin G residues in muscle were detected in 100% of samples at Day 1, but in no other groups. Injection site residues were detectable in at least one sow in all time points, with a 207.7 ng/g residue detected at Day 28. All raw tissue residue data are shown in Table 3. Sows administered the extralabel dose of PPG in TG3 had detectable residues in all tissue sample types. Kidney penicillin G residues were detected in 100% of sows at Day 1 and 50% at Day 6. One sow at Day 1 had measurable residues in the liver. Muscle residues were detected up to Day 14 postadministration and were found in 50% of sows sampled. Very high levels of residues were detected in the injection sites of the TG3. All sows at Day 1 and Day 6 necropsy time points had injection site residues. Only 25% of sows had injection site residues at Day 28. Sows in TG2 were injected only with sterile saline to serve as a negative control. No detectable residues were found in kidney, muscle, or liver tissues in any sow at any time point. Injection site residues were detected in five sows with at least one sow at each sampling time point.

3.2 | Comparison of available analytical methods

The LC-MS/MS methods used in current study were similar to methods used by the FSIS to detect penicillin residues. By using the more sensitive LC-MS/MS method, this study used lower LOQ of 5 ng/g for different tissues initially. Due to a large amount of variability seen at this level, the data were finalized using the FSIS standards. The FSIS reported LOQs for kidney, liver, and muscle at 15, 30, and 5 ng/g, respectively. The LOQ of 5 ng/g was used for plasma, and 30 ng/ml used for urine. The LODs of penicillin G for kidney, liver, and muscle are 0.2 ng/g, for plasma is 0.5 ng/g, and for urine is 5 ng/ml. The FSIS also uses the kidney inhibition swab (KIS) test to screen for residues. A comparison of the current LC-MS/MS results with the results of the KIS test on the same samples is listed in Table S3. In TG1, the LC-MS/MS method detected three more positive residue tests of kidneys than the KIS method. The two assays agreed completely with the TG2 controls. They correlated well in the extralabel TG3 sows, with both the LC-MS/MS and KIS tests detecting 100% of samples with residues at Day 1 and 50% of samples with residues at Day 6. However, samples that were tested positive at Day 6 were not the same sows. The LC-MS/MS testing methodology was consistent with the KIS testing of kidneys used by the FSIS and is a reliable analytical tool to assess penicillin G residues. Compared with LC-MS/MS method, the KIS test was less sensitivity and specificity

			Treatment group 3					
	Injection site						Injection site	
Muscle	(Dorsal)	(Ventral)	Sow ID	Kidney	Liver	Muscle	(Dorsal)	(Ventral)
<LOQ	<LOQ	<LOQ	447	19.4	<LOQ	98.2	177772	357204
<LOQ	<LOQ	<LOQ	441	38.8	67.1	72.6	12518610	N/A
<LOQ	98.7	555.4	444	44.7	<LOQ	115.7	1469383	2674478
<LOQ	<LOQ	<LOQ	453	679.9	<LOQ	59.6	460021	31689
<LOQ	<LOQ	<LOQ	450	64.9	<LOQ	21.7	436.5	3645.9
<LOQ	24.6	<LOQ	467	16.3	<LOQ	19.8	227.9	31667
<LOQ	<LOQ	185	455	<LOQ	<LOQ	<LOQ	53.1	527.6
<LOQ	<LOQ	<LOQ	346	<LOQ	<LOQ	22	53	90121
<LOQ	945.5	79.1	443	<LOQ	<LOQ	6.8	<LOQ	234.7
<LOQ	<LOQ	<LOQ	457	<LOQ	<LOQ	<LOQ	85.7	<LOQ
<LOQ	<LOQ	<LOQ	454	<LOQ	<LOQ	6.4	51.7	71.6
<LOQ	<LOQ	<LOQ	461	<LOQ	<LOQ	<LOQ	599.8	<LOQ
<LOQ	<LOQ	<LOQ	341	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
<LOQ	<LOQ	114.4	442	<LOQ	<LOQ	<LOQ	433	194.5
<LOQ	N/A	N/A	464	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
<LOQ	<LOQ	<LOQ	449	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

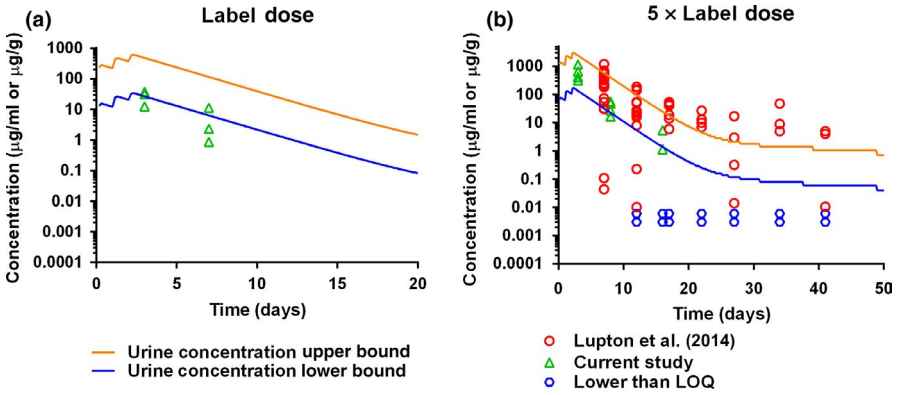

FIGURE 2 Simulation of urine concentration of penicillin G using the PBPK model. Comparison of model simulations (solid lines) and observed data (red circles, green triangles, and blue hexagons) for concentrations of penicillin G in urine of heavy sows exposed to PPG via repeated 3 doses of IM injections at label dose (6.5 mg/kg, [a]) and 5 × label dose (32.5 mg/kg, [b]). Experimental data (individual data points) of panel A are from current study, and experimental data of panel B are from the study of Lupton et al., 2014 and current study. The data points less than LOQ were marked with the 0.5-fold and 0.2-fold LOQ using blue hexagons for illustration purpose. Two hexagons indicate that there were 2 or more than 2 animals with concentrations lower than LOQ at a specific time point [Colour figure can be viewed at wileyonlinelibrary.com]

for penicillin G and produced more variability at levels lower than LOQ used by FSIS.

3.3 | Antemortem marker for penicillin G

Urine samples were analyzed by LC-MS/MS, SNAP, and Charm MRL tests. A complete list of urine analysis result is provided in Table S6. Mild discrepancies in positive test results among the three assays were mostly seen as time increased from cessation of PPG administration. This could be due to reduced sensitivity in the assays at low levels of quantitation. The SNAP and Charm MRL tests also are less specific than the LC-MS/MS and may be more likely to report false positive results. As a sample type, urine residues of penicillin G were found correlated to plasma and tissue residues. TG1 sows had urine residues at Day 6 postadministration of PPG and TG3 sows had urine residues at Day 14 postadministration of PPG when analyzed by

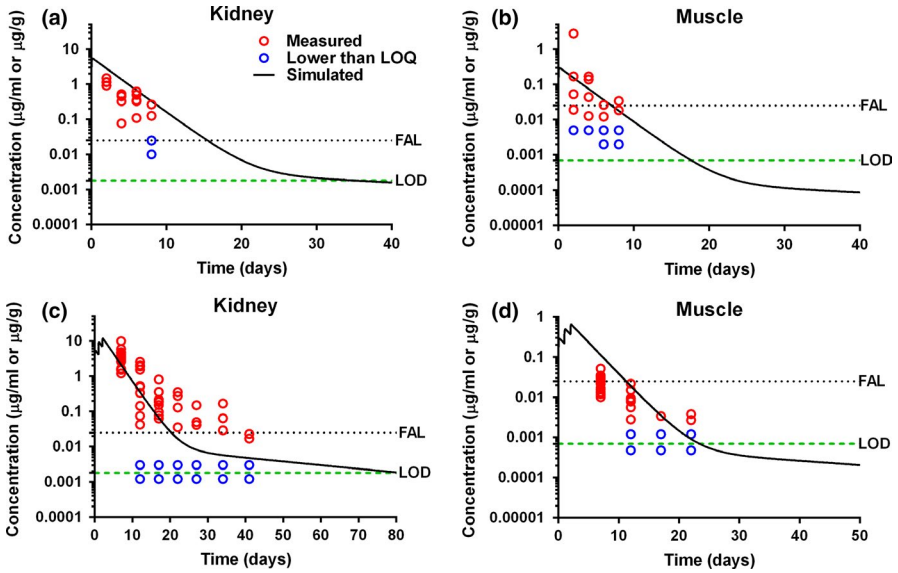

FIGURE 3 Calibration of the heavy sow PBPK model. Comparison of model simulations (solid line) and observed data (red circles) for concentrations of penicillin G in the kidney and muscle of heavy sows exposed to PPG via single IM injection (32.5 mg/kg, a,b), and repeated 3 doses of IM injections (32.5 mg/kg, c,d). Experimental data (individual data points) are from previous studies: panel a and b (Apley et al., 2009); panel c and d (Lupton et al., 2014). The data points less than LOQ were marked with the 0.5-fold and 0.2-fold LOQ using blue circles for illustration purpose. One blue circle indicates that there was one animal with penicillin G concentration lower than LOQ at a specific time point; two blue circles indicate that there were 2 or more than 2 animals with concentrations lower than LOQ at a specific time point. The values of LOQs are summarized in Table 1. The FSIS action limit (FAL) is shown using the dotted line. FAL for penicillin G in heavy sows is 25 ng/g (FSIS, 2013). The limit of detection (LOD) is shown in each panel using green dash line. LOD for the kidney is 1.8 ng/g and for the muscle is 0.7 ng/g (Lupton et al., 2014) [Colour figure can be viewed at wileyonlinelibrary.com]

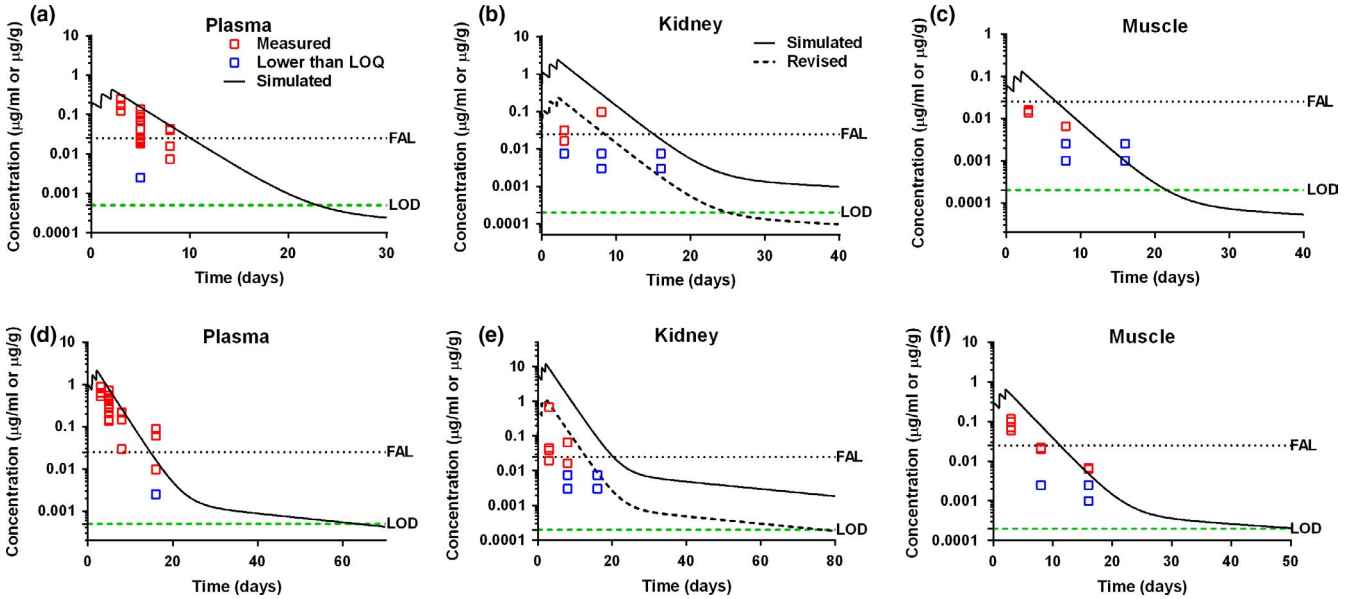

FIGURE 4 Evaluation of the heavy sow PBPK model with pharmacokinetic data from current study. Comparison of model predictions (solid line) and observed data (red squares) for penicillin G concentrations in the plasma, kidney, and muscle of heavy sows exposed to procaine penicillin G via IM repeated 3 doses at 6.5 mg/kg (a,b,c) and at 32.5 mg/kg (d,e,f) is shown. The data points less than LOQs were marked with the 50% and 20% LOQ using blue squares for illustration purpose. One blue square indicates that there was one animal with penicillin G concentration lower than LOQ at a specific time point; two blue squares indicate that there were 2 or more than 2 animals with concentrations lower than LOQ at a specific time point. The FSIS action limit (FAL) is shown using the dotted line. FAL for penicillin G in heavy sows is 25 ng/g (FSIS, 2013). The limit of detection (LOD) is shown in each panel using green dash line. LOD for the plasma is 0.5 ng/g, for the kidney is 0.2 ng/g, and for the muscle is 0.2 ng/g. The simulation results with the revised value of kidney partition coefficient are shown in panel B and E with black dash lines [Colour figure can be viewed at wileyonlinelibrary.com]

LC-MS/MS. Both rapid tests, Charm MRL and SNAP, had consistent results when detecting urine residues compared to the LC-MS/MS analysis. As the urine production rates from experimental studies were from 0.0003 to 0.012 L h^{-1} kg^{-1} (Chastain et al., 1999; Deding et al., 2006; Hamilton et al., 1997; Hannon et al., 1990; Patience et al., 1987), the upper and lower bounds of penicillin G concentrations in urine were calculated based on this range and shown in Figure 2. The PBPK model simulation predicted the urine concentrations (Figure 2) based on variable urine production rates after both label and extralabel doses. The lower bound of the penicillin G concentrations over predicted some of the data points with low penicillin G levels. This may be due to the very wide range of penicillin G concentrations measured in urine samples, especially for some urine samples after 25 days of penicillin G administration (Figure 2b). The pharmacokinetic profile of urine was very similar compared to other tissue samples.

3.4 | PBPK model calibration and evaluation

The simulated results for concentrations of penicillin G in plasma and edible tissues at different time points after administration were compared with observed concentrations in heavy sows exposed to penicillin G through single IM injection with the dose of 5 × label dose (32.5 mg/kg), and repeated IM injections of 32.5 mg/kg for 3 times (representative results are shown in Figure 3; other results are provided in Supporting information Figure S1). Overall, the model adequately captured the kinetic profiles of penicillin G in different edible tissues and plasma in heavy sows (Figure 3 and Supporting information Figure S1). The model in general properly predicted the penicillin G concentrations in muscle (Figure 3b,d), but over predicted the first time point after repeated IM injections (Figure 3d). For penicillin G concentrations in kidney, the model adequately simulated the multiple-dose scenario (Figure 3c) and slightly over predicted the single IM injection treatment (Figure 3a). The overall determination coefficient (R^2) of linear regression analysis for calibration data sets was 0.85 (Supporting information Figure S2b).

The pharmacokinetic data from the current study were used to evaluate the performance of the PBPK model for heavy sows. Measured concentrations of penicillin G in edible tissues of heavy sows after IM injections of 6.5 mg/kg (Figure 4 a,b,c) or 32.5 mg/kg (Figure 4 d,e,f) for 3 consecutive days were compared with model predictions. The model slightly over predicted the early phase for plasma, liver, and muscle (within twofold difference in Figure 4 and Supporting information Figure S2), and greatly over predicted the concentrations of penicillin G in kidneys for both doses (Figure 4b,e). The exact reason for this over prediction is unknown, but it may be because the measured concentrations in kidney in the present study were fairly low compared to previous two studies (Apley et al., 2009; Lupton et al., 2014). By adjusting the kidney partition coefficient value from 10 to 1, the simulation (blacked dashed lines in Figure 4b,e) better correlated with the measured data for both doses. In the present model, we kept

the originally calibrated partition coefficient for kidney, but additional studies to measure this parameter value experimentally are needed to improve this model. The over predictions of the model simulation were also reflected in the regression analysis presented in Supporting information Figure S2c, and more data points fell below the line of equality, which means simulated values are larger than observed values. Other model evaluation results are presented in Supporting information Figure S2.

3.5 | Sensitivity analysis

The local sensitivity analysis was carried out for 25 model parameters based on the PBPK model for heavy sows. Results of the local sensitivity analysis based on 1% variation of the parameter values are shown in Table S7. Only parameters with at least one absolute value of normalized sensitivity coefficients (NSCs) greater than 0.05 are shown in the table. All the selected area under the curves (AUCs) were highly sensitive to intramuscular absorption rate constant (Kim) with the NSC value of 0.83. The AUC of liver was highly sensitive to liver partition coefficient (PL) with the NSC value of 0.94. The AUC of kidney was highly sensitive to urine elimination rate constant (KurineC) and kidney partition coefficient (PK) with NSC values of −0.95 and 1.00, respectively. The AUC of muscle was highly sensitive to muscle partition coefficient (PM) with the NSC of 0.99. Among the highly sensitive parameters, Kim, PL, PK, and PM were designated with high uncertainty, others had low or medium uncertainty.

3.6 | Determination of withdrawal intervals (WDIs) in heavy sows

The population PBPK model was used to estimate the WDIs after label or extralabel use of penicillin G in heavy sows. The kidney tissue residue depletion profiles for heavy sows were used to determine the WDIs. The label withdrawal periods were obtained from the Veterinarian's Guide to Residue Avoidance Management (VetGRAM) of FARAD (Riviere, Tell, Baynes, Vickroy, & Gehring, 2017), and they are highly dependent on specific drug formulations. The label withdrawal periods of PPG (in NADA: 650–174) for swine are 6 days. Based on the FSIS action limit, the model-predicted WDI after single IM injection with label dose 6.5 mg/kg was 22 days, and the model-predicted WDI following 3 repeated IM injections at 32.5 mg/kg in heavy sows was 38 days (Figure 5). The results of population analysis overlaying with available pharmacokinetic data are shown in Supporting information Figure S3. The predicted WDI with label dose after single IM injection is conservative compared to label withdrawal period of 6 days in swine (all use classes). The predicted WDI after 3 repeated IM injections at 5x label dose is also conservative compared to reported WDI of 28 days in heavy sows from Apley et al. (Apley et al., 2009) and is shorter than 51 days for heavy sows estimated in the study of Lupton et al (Lupton et al., 2014). Note that if the estimated WDI was a fraction of a day, it was rounded up to the next whole day. By using 22 days as the WDI for label dose in the withdrawal interval calculator (Gehring, Baynes, Craigmill, & Riviere, 2004), the predicted WDI for extralabel dose of 32.5 mg/kg from the calculator was 40 days, which is very close to the predicted WDI from the current PBPK model. If 6 days were used as the withdrawal period for label dose in heavy sows, the predicted WDI using the withdrawal interval calculator for extralabel dose 32.5 mg/kg was only 11 days. Therefore, the WDI for extralabel dose calculated based on withdrawal period from market-age swine may not be protective for heavy sows from the food safety perspective. Due to the differences between market-age swine and heavy sows, determination of the withdrawal period of penicillin G in heavy sows only based on the pharmacokinetic data from market-age swine may not be appropriate.

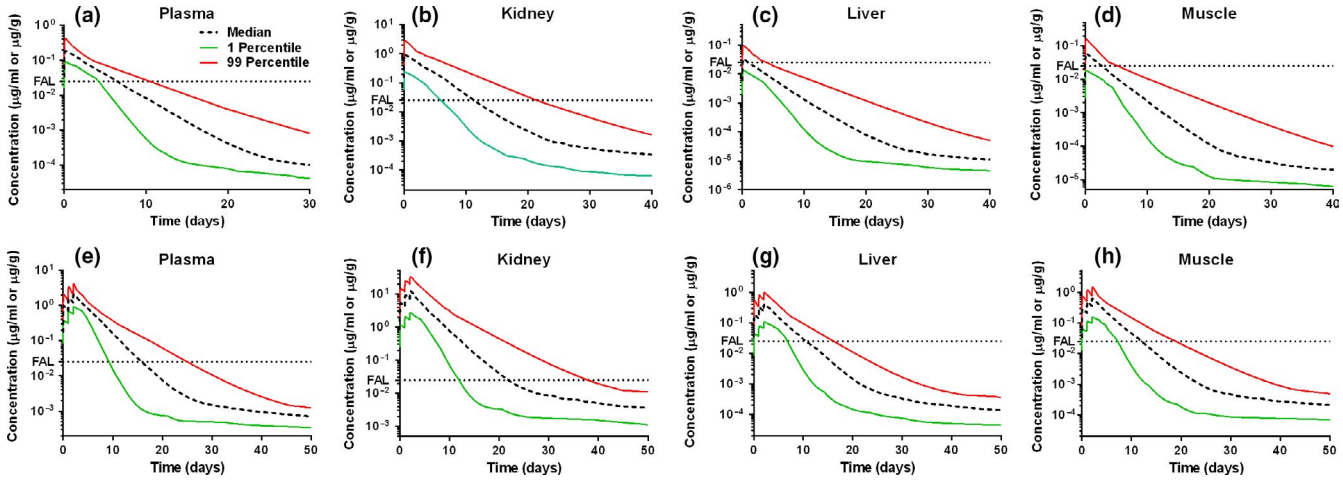

FIGURE 5 Monte Carlo simulations of penicillin G concentrations in plasma, kidney, liver, and muscle using the population PBPK model in heavy sows. The label dose of 6.5 mg/kg after single IM injection (a–d) and the commonly used extralabel dose (5 × label dose, 32.5 mg/kg) with 3 repeated IM injections (e-h) were simulated as the therapeutic scenarios for heavy sows. Each of the simulations was run for 1,000 iterations. The median, 1st and 99th percentiles of simulated results were plotted. The FSIS action limit (FAL) is shown on each of panels using the dotted line. The extended withdrawal intervals were determined when the tissue concentrations of penicillin G fall below FAL for the 99th percentile of the population [Colour figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

In this study, the plasma pharmacokinetics and residue depletion in edible tissues of PPG in heavy sows were determined. Tissue, plasma, urine, and environmental samples were analyzed with three different methods including LC-MS/MS, SNAP, and Charm MRL tests. The results suggest that the urine samples have the potential to be the antemortem marker for penicillin G in heavy sows. The PBPK model for PPG in heavy sows was developed with all available pharmacokinetic data based on the previous generic PBPK model for PPG in swine and cattle (Li et al., 2017). The present population PBPK model could be used to predict tissue concentrations and withdrawal intervals following extralabel use of penicillin G in heavy sows.

In the last decade, PBPK modeling has been widely used in the area of veterinary medicine, from the prediction of drug tissue residues (Huang et al., 2015; Leavens et al., 2012; Yang et al., 2018), estimating the withdrawal time (Buur, Baynes, Smith, & Riviere, 2006; Yang, Huang, et al., 2014; Yang, Zhou, et al., 2015), to facilitating the food safety assessment (Henri, Carrez, Meda, Laurentie, & Sanders, 2017; Lin, Gehring, Mochel, Lavé, & Riviere, 2016; Yang, Huang, et al., 2014; Yang, Zhou, et al., 2015). In this study, a PBPK model was established for heavy sows based on a previous generic PBPK model for swine and cattle (Li et al., 2017). Overall, the PBPK model properly predicted the majority of available plasma and tissue concentration data of penicillin G and over predicted the kidney concentrations from the current study. Based on the simulation results, there is an apparent discrepancy in the reported tissue depletion profiles of penicillin G in heavy sows among existing studies. The exact reasons for this discrepancy are unknown, but it could be due to the differences in the management, nutritional strategies, breed, and age variability of heavy sows among different studies. Specifically, it has been reported that heavy sows are quite variable in term of hormones (Oliviero, Heinonen, Valros, Halli, & Peltoniemi, 2008), heat stress tolerance (Bloemhof, Waaij, Merks, & Knol, 2008), and behaviors (Broom, Mendl, & Zanella, 2010), due to genetic differences and different environmental factors. The differences may be also caused by sample handling across different laboratories. As most of the penicillin G will be in the cortex where tubular secretion occurs, any urine contamination would lead to additional variances. In addition, it may be also due to the intrinsic high variability in the physiological characteristics among different populations of heavy sows. Also, different sensitivities or random experimental errors between different measurement methods among these studies may lead to the study differences. Different approaches were tried to improve the model fit. Based on the sensitivity analysis result and after adjusting the kidney partition coefficient (PK) value from 10 to 1, the predictions were improved (blacked dashed lines in Figure 4b,e). More residue depletion and pharmacokinetic studies of penicillin G in heavy sows are needed to investigate the reasons of the discrepancy among existing studies and to further improve the current PBPK model.

Based on the results from tests of potential antemortem markers, the antemortem urine testing with either Charm MRL or SNAP tests would provide potential information about penicillin G residues in the sow of interest. In addition, whenever possible, field testing of residues in urine samples should be conducted. At low plasma concentrations, other weak acids secreted by renal tubules may compete with penicillin G excretion. In addition, other factors, such as disease conditions, management, and nutritional strategies, and co-administration of other drugs, could affect penicillin urinary excretion, but these factors were not included for model simulations.

Many of the detectable injection site residues were found at very high levels for sows in TG1 and TG3, and some were even over 1 million ng/g of tissues. High levels of penicillin G in the injection sites at all time points could be due to the large injection volume and slow distribution of penicillin G through the tissues. In addition, sows have a thick layer of fat overlaying the hip region where injections were given. A one-inch needle may not have penetrated the muscle in more well-conditioned sows and some of the high levels of residues could be due to penicillin G that is bound in the fat layer. Penicillin G tissue residue depletion appears to be concentration-dependent. As the concentration increased, a higher percentage of sows had tissue residues in all four tissues sampled. Additionally, the concentrations of penicillin G residues found in TG3 were on average higher than TG1. Based on these data, tissue residues can be found in sows administered extralabel doses at least 28 days postadministration. This is much greater than the estimated 15-day withdrawal interval from the Korsrud et al. study (Korsrud et al., 1998).

The sensitivity analysis of the current PBPK model indicates the uncertainties of some parameters have influences on the predictions of the concentrations of penicillin G in heavy sows. The results also indicate that the variations of physiological parameters have relatively less impact on model simulations. The partition coefficients of muscle, liver, and kidney were highly influential on the prediction of the tissue concentrations. These parameter values were estimated based on available pharmacokinetic data sets. The values of tissue to plasma partition coefficients in this study are slightly different from the values in the previous PBPK model for market-age swine (Li et al., 2017). This is mainly because values of partition coefficients for market-age swine were estimated by fitting to market-age swine tissue data, whereas values in the present study were re-estimated by fitting to available heavy sow tissue data. The potential reasons for the discrepancies could be because of the higher fat components in these tissues of heavy sows compared to the market-age swine. Overall the liver, kidney, and muscle partition coefficients in heavy sows were comparable to the experimental values from a previous PBPK model in rats (Tsuji et al., 1983) and the values in our previous PBPK model in market-age swine (Li et al., 2017). However, additional studies that determine the values of partition coefficients in different tissues of swine using experimental methods (Pacifici & Viani, 1992; Tremblay, Kim, & Fisher, 2012) are needed. The intramuscular absorption rate constant in heavy sows is comparable (within five-fold difference) to the model-fitted values used in the previous PBPK model for swine and cattle (Li et al., 2017). The difference may be due to the higher fat components under skin in heavy sows

and the reduced capillary vascular density, which lead to the lower intramuscular absorption rate constant. The urine clearance rate constant also has impacts on the model predictions. The urinary clearance rate of heavy sows (0.8 L h^{-1} kg^{-1}) is comparable (within twofold difference) with the reported clearance rates in sheep (0.55 L h^{-1} kg^{-1}) and horses (0.51 L h^{-1} kg^{-1}) (Firth, Nouws, Klein, & Driessens, 1990; Oukessou, Hossaini, Zine-Filali, & Toutain, 1990; USP, 2007), but it is lower than the urinary clearance rate of 1.4 L h^{-1} kg^{-1} for market-age swine (Li et al., 2017). The ~ twofold difference in urinary clearance between heavy sows and market-age swine may contribute to the predicted longer withdrawal interval for penicillin G in the former than in the latter.

The extended WDI for extralabel use of penicillin G were determined based on the simulation results from probabilistic models against FAL. The current model was calibrated using experimentally measured pharmacokinetic data above and below FAL. However, if zero tolerance is established for veterinary medicines, in operation, LOD would be used to determine withdrawal intervals. The actual pharmacokinetic data below LOD would not be available, which may lead to uncertainties of the model prediction. For example, due to saturable kinetics in the absorption, uptake and especially tubular secretion and elimination, the lower doses may lead to higher absorption and altered rates of elimination (Lin, Fisher, Ross, & Filipov, 2011; Teeguarden, Dorman, Covington, Clewell, & Andersen, 2007). In addition, the reversible protein binding of drugs may lead to the increase of free drug concentrations at low concentrations (Bohnert & Gan, 2013). The protein binding of penicillin G in the plasma of swine was reported as 36.6% (Keen, 1965). All these required extra attentions for model predictions at low plasma or tissue concentration levels, where no measured pharmacokinetic data are available.

The population PBPK model can be a useful tool to predict the tissue concentrations and withdrawal intervals following extralabel use of veterinary drugs (Henri et al., 2017; Lin, Gehring, et al., 2016; Yang, Huang, et al., 2014; Yang, Yang, et al., 2014). The current model provides a conservative estimation of extended withdrawal intervals based on all available pharmacokinetic data of penicillin G in heavy sows and significantly extends label-recommended withdrawal periods. However, due to very limited drug depletion studies for penicillin G in heavy sows available and the high variabilities among the available data sets, the model still needs be improved with additional studies to better predict the tissue concentrations and WDIs. The variabilities from different sources should be considered, and more mechanistic studies should be carried out to help better understand the differences among species and breeds (Martinez, Court, Fink-Gremmels, & Mealey, 2018; Martinez, Gehring, Mochel, Pade, & Pelligand, 2018). As the depletion studies were carried out by different labs and with different commercial brands of PPG, the experimental and random variances may be larger than the physiological variability considered in the population PBPK model. The population analysis in current PBPK model did not account for the variabilities between different studies, which could potentially be addressed using the nonlinear mixed-effect population pharmacokinetic modeling approach (Bon et al., 2018; Li, Gehring, Lin, & Riviere, 2015; Mochel et al., 2013; Mould & Upton, 2012,2013; Riviere, Gabrielsson, Fink, & Mochel, 2016).

There were several limitations for the present study. One limitation was the relatively limited studies available for penicillin G tissue depletion in heavy sows. Among the three available studies for penicillin G in heavy sows (Apley et al., 2009; Lupton et al., 2014; the current study), there was an obvious discrepancy in the reported tissue depletion profiles. As a result, while the present PBPK model was properly calibrated with published studies, it over predicted the observed data collected as a part of the current study. The reason for the discrepancy is still unknown; and the impact of this over prediction on the estimated withdrawal interval remains to be investigated. Also, in the present pharmacokinetic study, there were a few samples with undetectable concentrations at the terminal phase. Drug residue data at the terminal phase is critical in the determination of withdrawal periods. In the future, a more sensitive method needs to be developed to measure the drug concentration of penicillin G at the terminal phase. In addition, the current PBPK model cannot simulate the variances of the management and nutritional strategies for food animals, and these differences may lead to significant differences of drug concentrations in edible tissues. Additional studies of penicillin G tissue depletion in heavy sows are needed to improve the present model and to determine the potential reasons for the discrepancy among different studies.

5 | CONCLUSION

The drug depletion and pharmacokinetic study for penicillin G in edible tissues and plasma following the label and extralabel doses of PPG helps us better understand the disposition and elimination of penicillin G in heavy sows. By using all currently available pharmacokinetic data, a PBPK model for PPG was developed specifically for heavy sows, and the model adequately simulated most of the observed penicillin G concentrations in edible tissues and plasma. Based on the model simulation results, urine samples have the potential to be the antemortem marker for penicillin G in heavy sows. Furthermore, the population PBPK model with Monte Carlo analysis could be used to predict tissue concentrations and withdrawal intervals following extralabel use of penicillin G in heavy sows. This study also suggests that it is feasible to extrapolate PBPK models across ages or across different use classes of food-producing animals. Future drug depletion studies for penicillin G in heavy sows are needed to figure out the potential reasons for the discrepancy among available studies and to improve the current model.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

JFC, LAK, and CM designed the residue depletion experiments. CM, LWW, LAK, and JFC conducted the residue depletion experiments and analyzed the plasma, tissue, urine, and environmental samples. ZL, ML, JER, and RG designed the PBPK model. ML developed the PBPK model, ran all simulations, and analyzed the model simulation results under the mentorship of ZL. DZ contributed to PBPK model development. All authors contributed to data interpretation. ML, CM, and ZL wrote the manuscript. All authors have read and approved the final manuscript.

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SUPPORTING INFORMATION

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