DOI:10.1021/jf101012r

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Distribution of Penicillin G Residues in Culled Dairy Cow Muscles: Implications for Residue Monitoring

Marilyn J. Schneider,*,† Katerina Mastovska,†,‡ and Morse B. Solomon§

†Eastern Regional Research Center, Agricultural Research Service, United States Department of Agriculture, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, and §Beltsville Area Research Center, Building 209, Agricultural Research Service, United States Department of Agriculture, 10300 Baltimore Avenue, Beltsville, Maryland 20705. ‡Current address: Covance Laboratories, Nutritional Chemistry and Food Safety, 671 S. Meridian Road, Greenfield, IN 46140.

The U.S. Food and Drug Administration sets tolerances for veterinary drug residues in muscle but does not specify which type of muscle should be analyzed. To determine if antibiotic residue levels are dependent upon muscle type, seven culled dairy cows were dosed with penicillin G (Pen G) from 1 to 3 days and then sacrificed on day 1, 2, or 5 of withdrawal. A variety (9−15) of muscle samples were collected, along with liver and kidney samples. In addition, corresponding muscle juice samples were prepared. All samples were extracted and analyzed by liquid chromatography−tandem mass spectrometry (LC−MS/MS) to determine Pen G levels. Results showed that Pen G residue levels can vary between and within different muscles, although no reproducible pattern was identified between cows or withdrawal times. Muscle juice appeared to be a promising substitute for muscle as a matrix for screening purposes. Because of the potential for variation within muscles, all samples taken need to be large enough to be representative.

KEYWORDS: Penicillin G; muscle; residue; distribution; monitoring

INTRODUCTION

The U.S. Food and Drug Administration (FDA) regulates the use of veterinary drugs in food-producing animals and sets tolerances for drug residue levels in animal-derived food products. Monitoring of these food products is performed to ensure that violative levels of drug residues are not present in the food supply. Tolerances are nearly always set for muscle; however, no particular muscle is specified for monitoring purposes.

Studies have indicated that drug residue levels can vary within a kidney (*1*, *2*), but little is known about distribution of these residues between or within different muscles. In poultry, two studies have shown differences in drug residue levels between breast and thigh muscles. Initial residue levels of the coccidiostat diclazuril were found to be higher in chicken thigh muscle than in breast muscle, with the authors speculating that this may be due to higher blood flow in the thigh (*3*). Levels of enrofloxacin, however, were found to be higher in chicken breast muscle than in thigh muscle (*4*). A study of carazolol determination in pork tissue sampled two muscle tissues 1−2 h post-injection and found carazolol in the diaphragm but not in gluteal muscle (*1*). Finally, six muscles from three calves dosed with tilmicosin were investigated 72 h post-treatment. Considerable variation was observed in tilmicosin levels between muscles, and subsampling showed a high degree of variation within individual muscles as well (*5*).

In this work, we determined the distribution of penicillin G (Pen G) in 9−15 different muscles of culled dairy cattle using a recently developed liquid chromatography−tandem mass spectrometry (LC−MS/MS) method (*6*). Pen G was chosen for this study because it is a commonly used veterinary drug and is approved for use in cattle (*7*, *8*). Two portions of each selected muscle were sampled to further determine if variation existed within each muscle. In addition, we produced muscle juice by squeezing subsamples of selected muscles, to determine if the easier to work with muscle juice could be used as a matrix for effective screening and/or determination of Pen G levels in muscle.

*To whom correspondence should be addressed. Telephone: (215) 233-6743. Fax: (215) 233-6642. E-mail: marilyn.schneider@ars.usda.gov.

MATERIALS AND METHODS

Reagents and Materials. Pen G and Pen V standards were obtained from U.S. Pharmacopeia (Rockville, MD); formic acid (98%) was from Fluka (Buchs, Switzerland); and C_{18} sorbent (40 μm) was from JT Baker (Phillipsburg, NJ). Acetonitrile was obtained from Mallinckrodt (Phillipsburg, NJ) and was LC/UV-spectrophotometric-grade. Control beef semitendinosus (ST) muscle was purchased from a local store, and control beef kidney was obtained from a local beef processing plant. Both types of control beef samples were analyzed by LC−MS/MS to ensure that Pen G and Pen V were absent prior to their use as controls in this study. All aqueous solutions were prepared with water from a Barnstead purification system (Dubuque, IA).

Standard Solutions. Standard aqueous stock solutions of Pen G (1550 μg/mL) and Pen V (560 μg/mL) were prepared and stored as aliquots in vials at −20 °C. Dilutions of these stock solutions were prepared for each day's experiment and used for calibration curve and fortified samples.

Incurred Tissues. Culled dairy cows, ranging from approximately 4−7 years in age, were injected in the ST muscle with Pen G Procaine aqueous suspension (300 000 units/mL, Hanford US Vet. Products) on

 Published on Web 04/12/2010

3 consecutive days (30, 20, and 20 mL, respectively), except for cow 1, which received one dose of 15 mL. The animals were sacrificed by the captive bolt method after 1, 2, or 5 days of withdrawal, and tissue samples were transported to the laboratory in dry ice and then stored at −80 °C. One kidney from each animal was homogenized with a food processor (Robot coupe, Jackson, MS), and a portion (100 g) of liver from each animal as well as a portion (100 g) of each muscle sample was homogenized with a small food processor. The individual homogenized samples were stored at −80 °C until analysis. Five smaller portions of each muscle sample (20 g) were transferred to small plastic bags and pressed in a vise to produce muscle juice, which was then stored at −80 °C until analysis. Muscle samples collected from each animal include anterior (ANT) and posterior (POST) portions of cruspillar, hanging tenderloin (HT); sternomandibularis, neck (NK); ST; and foreshank (FS); and dorsal (DOR) and lateral (LAT) portions of vastus lateralis (VL); longissimus dorsi, ribeye, and short loin (LD); supra spinatis, chuck tender (SS); semimembranosus, top round (SM); as well as tongue (TG). Additional samples collected from cows 1 and 2 included ANT and POST portions of biceps femoris, bottom round (BF); triceps brachii, shoulder clod (TB); and infra spinatis, flatiron (IS); and DOR and LAT portions of rectus femoris (RF); vastus intermedius (VI); and vastus medialis (VM) (**Figure 1**).

Extraction of Pen G. Pen G was extracted from muscle, kidney, and liver samples using a previously validated multi-residue method for β-lactams (*6*). Muscle, kidney, or liver samples (1.0 g) were placed in 50 mL disposable centrifuge tubes. For fortified samples, an appropriate volume of Pen G stock dilution was added at this time. An appropriate amount of Pen V internal standard was then added, followed by 4:1 (v/v) acetonitrile/water (8 mL for muscle and 10 mL for kidney or liver). Muscle samples were homogenized (Ultra-Turrax, Janke and Kunkel, Cincinnati, OH), and the homogenizer probe was rinsed with 2 mL of 4:1 acetonitrile/water. Kidney or liver samples were vortex-mixed (30 s). After centrifugation (10 min, 3716*g*, Sorvall Legend RT, Thermo Fisher Scientific, Waltham, MA), the supernatants were decanted into a 15 mL disposable centrifuge tube containing C_{18} sorbent (0.5 g), vortexed (15 s), and then centrifuged (5 min, 3716*g*). A portion (5 mL) of the supernatant was pipetted into a 15 mL conical glass centrifuge tube and evaporated under nitrogen with a TurboVap LV (Zymark, Hopkinton, MA) to < 1 mL volume. Water was added to give a 1 mL volume, and the samples were then transferred to Mini-Uniprep syringeless filter autosampler vials [0.45 μm polyvinylidene difluoride (PVDF), Whatman, Florham Park, NJ].

Extraction of muscle juice followed the above procedure for kidney or liver, except that a 0.5 mL sample was extracted using 5 mL of 4:1 acetonitrile/water, 0.25 g of C_{18} was used, and 2.5 mL of final supernatant was evaporated to a volume of < 0.5 mL prior to taking up to 1 mL volume and transfer to a Mini-Uniprep autosampler vial. Fortification was accomplished by the addition of an appropriate volume of Pen G stock dilution to the muscle juice prior to extraction.

LC−MS/MS Analysis. An Agilent 1100 high-performance liquid chromatography (HPLC) system including a binary pump, autosampler, column heater (30 °C), and degasser (Agilent, Palo Alto, CA) was coupled to an API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Toronto, Ontario, Canada). A 2.0 × 50 mm Prodigy ODS-3 column with a 2.0 × 4 mm C_{18} guard column (Phenomenex, Torrance, CA) was used, and the injection volume was 10 μL. Mobile phases consisted of A, 0.1% aqueous formic acid; and B, 0.1% formic acid in acetonitrile. Analytes were eluted with a linear gradient beginning with 20% B (0−1.5 min) and going to 100% B (2.0−4.0 min), with a flow rate of 0.4 mL/min. Retention times for Pen G and Pen V were 3.28 and 3.32 min, respectively. A divert valve (Valco, Houston, TX) directed the column effluent into waste before and after Pen G and Pen V elution. The mass spectrometer was operated in positive electrospray mode, and two transitions were monitored for each analyte: Pen G, m/z 335 → 160 and 335 → 176; Pen V, m/z 351 → 160 and 351 → 114. Mass spectrometer operating conditions were as follows: dwell time, 50 ms; entrance potential, 10 V; ion spray voltage, 4500 V; ion source temperature, 525 °C; and curtain gas, 40 psi, with optimum setting of 11 in Analyst software. For Pen G and Pen V, the following values were optimum, respectively: declustering potential, 36 and 21 V; focusing potential, 180 and 110 V; collision energy for the two transitions, 17 and 17 V and 17 and 45 V; and collision cell exit potential for the two transitions, 14 and 12 V and 14 and 8 V.

RESULTS AND DISCUSSION

Determination of Pen G. Pen G levels were determined using a recently developed LC−MS/MS method (*6*). External calibration curves in water were linear from 0.2 to 200 ng/g Pen G ($r^2 \geq 0.998$). A comparison of the external calibration curves to matrix-matched calibration curves in muscle, muscle juice, kidney, and liver extracts indicated significant matrix suppression only in liver and kidney samples. Pen G levels in these extracts required a 1:100 dilution with water, which also served to practically eliminate the matrix suppression (reduced to a level of +5% for liver and +1% for kidney). Thus, external calibration curves in water were found to be adequate for the study. The lowest calibration level (LCL) was 0.2 ng/g Pen G, which served as the reporting limit. Recoveries of undiluted samples fortified at 20−40 ng/g Pen G were good, ranging from 86 to 111% (muscle juice) and 94−119% (muscle). Good recoveries for liver and kidney samples fortified at 1000−2000 ng/g and then diluted 1:100 were also obtained, ranging from 98 to 105% (liver) and from 105 to 116% (kidney).

Distribution of Pen G Residues between Different Muscles, Liver, and Kidney. The first two cows studied were sacrificed after 1 day of withdrawal, and 15 muscles were sampled from each, along with liver and kidney. The locations of the sampled muscles are illustrated in **Figure 1**. The distribution of Pen G in these muscle samples is illustrated in **Figure 2**. The injection site (ST) and SM muscles are omitted from **Figure 2** because of their high levels of Pen G.

With all cows after the first two, a set of 9 of the original 15 muscles were selected for sampling, along with liver and kidney. Cow 3 was sacrificed at day 5 of withdrawal, and no Pen G was detected in any of the muscle, muscle juice, liver, or kidney samples, including the injection site. Cows 4−6 were sacrificed and sampled at day 2 of withdrawal, and cow 7 was, again, a day 1 of withdrawal example.

Data for Pen G levels in the 9 muscle samples common to all cows are summarized for withdrawal day 1 and withdrawal day 2 in **Tables 1** and **2**, respectively. Data for Pen G levels in liver and kidney are shown in **Table 3**. Upon examination of these tables of data, one thing that clearly stands out is the considerable variation that exists between cows, even in those subjected to the same dosing/withdrawal regimen. For example, cow 5 displays significantly lower levels of Pen G than the other two cows with the same (2 day) withdrawal time. Furthermore, the relative pattern of Pen G distribution between muscles is not always reproducible between animals. This phenomenon may be due, in part, to the variability in health and metabolism among these relatively old, culled cows. It is interesting to note, however, that similar variation was seen in the earlier study of tilmicosin in three identically treated calves (*5*).

Despite the observed variability, some patterns can still be seen in these data. First, Pen G levels in the liver, kidney, and injection site muscle (and juice) are relatively high. Second, levels of Pen G are generally lower in day 2 withdrawal samples than those from day 1 withdrawal. The exception to this is the injection site (ST) and sometimes muscles that are near the injection site, such as SM. While there may be differences between muscles within each cow, these patterns are, for the most part, not reproducible between cows. In general, however, HT and TG tend to contain among the higher muscle levels of Pen G outside of the injection site and LD contains among the lower levels, particularly among day 1 withdrawal samples.

Even on day 1 of withdrawal, the Pen G levels in the majority of muscles were below the U.S. tolerance of 50 ng/g for edible tissue. The exception is the muscles close to the injection site, which showed up to 65 000-fold exceedance of the tolerance level.

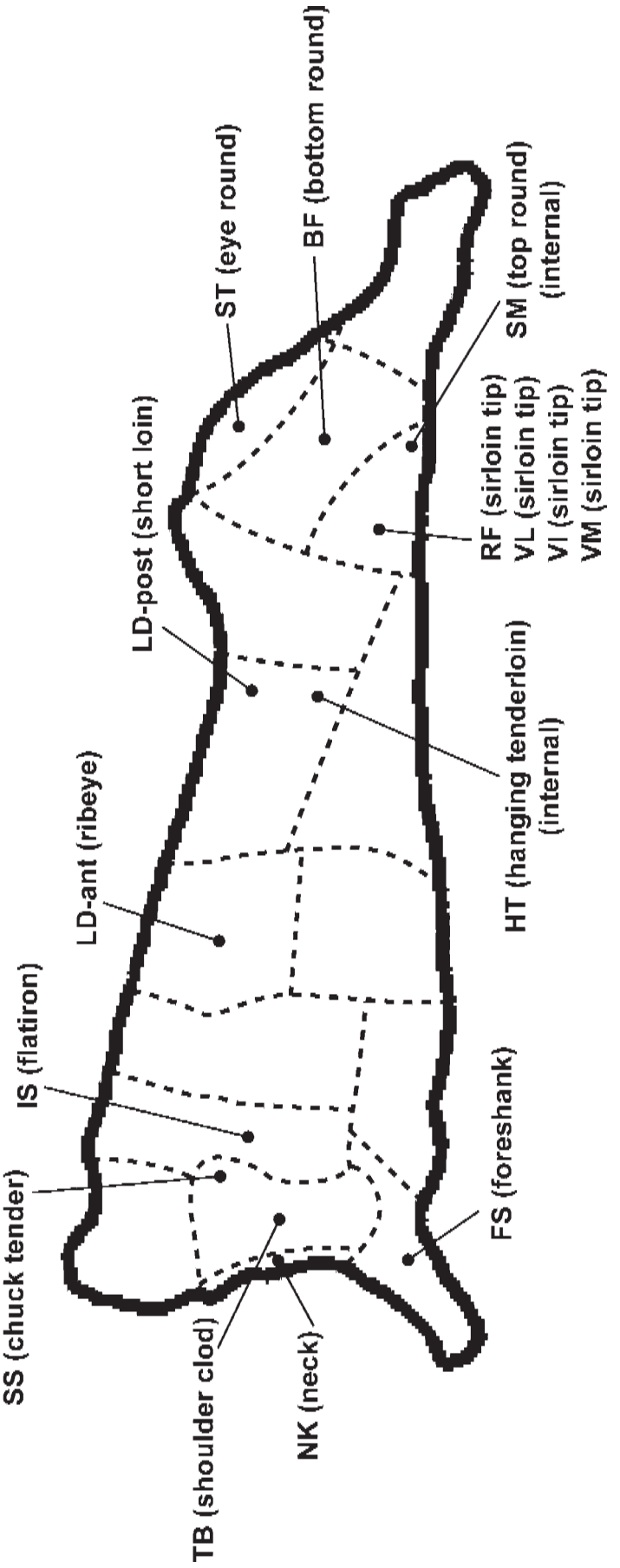

Figure 1. Location of bovine muscles sampled for the study. TG is not shown.

This can cause a serious acute health risk in the case of sensitive individuals. For this reason, an injection site should be targeted for veterinary drug residue screening in practice.

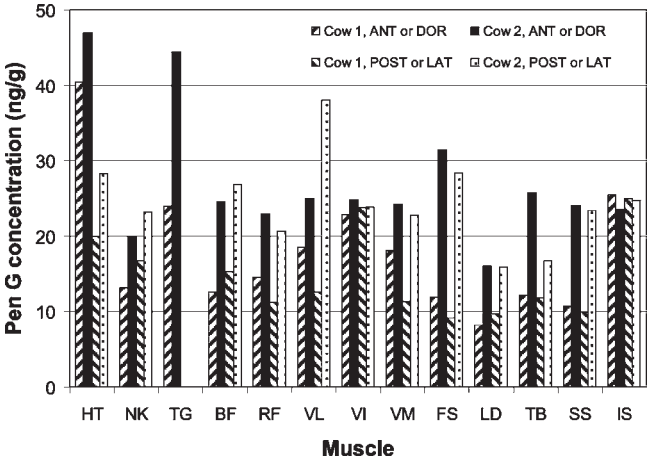

Figure 2. Comparison of Pen G levels within and between muscles ($n = 2$), withdrawal day 1, cows 1 and 2, ST and SM omitted. See **Figure 1** for muscle identification.

Distribution of Pen G Residues within a Muscle. Examination of the data in **Tables 1** and **2** shows where the two sampled portions of a muscle (ANT versus POST or DOR versus LAT) differ in Pen G levels. An example can be seen in **Table 1**, where HT-ANT values for Pen G are clearly higher than for HT-POST in cows 1 and 2, with the difference being as much as a factor of 2. The final cow in the table, cow 7, showed the same pattern, although to a lesser extent, with HT-ANT versus HT-POST muscles containing 33 versus 27 ng/g. The extent of these differences thus appears to be unpredictable. Further indications of differences within muscles can be seen in the data for muscle juice. Muscle subsamples (typically five of 20 g each) were taken from a given muscle to produce juice samples, which were then evaluated for Pen G levels and variability. These subsamples, while smaller than the 100 g portions homogenized for muscle analysis, were larger than those typically used for muscle juice analysis in the commercially available, Association of Official Analytical Chemists (AOAC) International validated Premi® Test (~2 cm^3) (*9*). The Pen G values in these juice samples displayed considerable variation within a set. This result is probably due to the muscle samples used to produce the juice samples being relatively small compared to those homogenized for muscle analysis (20 g rather than 100 g) and thus not fully representative of the overall muscle.

Table 1. Pen G Muscle and Muscle Juice Levels:[a] Withdrawal Day 1

	cow 1			cow 2			cow 7		
	muscle		muscle juice	muscle		muscle juice	muscle		muscle juice
sample	ng/g	average ng/g	average ng/mL (% RSD)	ng/g	average ng/g	average ng/mL (% RSD)	ng/g	average ng/g	average ng/mL (% RSD)
HT-ANT	41	40	24 (43)	46	47	88 (76)	35	33	37 (52)
	39			48			32		
HT-POST	19	20		27	28		26	27	16 (45)
	21			29			28		
NK-ANT	13	13		22	20	24 (26)	47	48	33 (61)
	13			18			48		
NK-POST	16	17		24	23	24 (3.7)	23	23	26 (33)
	17			22			24		
TG-TIP	23	24		45	44	86 (56)	170	180	290 (35)
	25			44			180		
ST-ANT[b]	13	13		1300	1300	29000 (130)	180	190	2700 (130)
	12			1300			190		
ST-POST[b]	11	11		2700000	3200000	380000 (200)	180	190	620 (130)
	12			3800000			200		
SM-DOR	17	16	38 (94)	840	850	770 (82)	120	120	210 (57)
	15			860			110		
SM-LAT	7.2	7.5	14 (36)	900	940	900 (42)	54	55	76 (82)
	7.8			990			55		
VL-DOR	18	19		26	25	25 (25)	42	41	45 (100)
	20			24			40		
VL-LAT	12	13		38	38	44 (24)	14	13	18 (40)
	13			39			12		
FS-ANT	12	12		30	31	32 (7.7)	78	78	54 (84)
	11			33			77		
FS-POST	8.7	9.2		29	28	28 (8.3)	15	16	18 (23)
	9.8			28			17		
LD-ANT	8.0	8.2	7.2 (6.2)	16	16	20 (6.9)	4.5	4.6	7.1 (70)
	8.3			17			4.6		
LD-POST	9.5	9.8		14	16	20 (11)	8.2	8.1	7.5 (67)
	10			17			8.0		
SS-ANT	11	11		25	24	25 (15)	9.4	11	36 (140)
	11			24			12		
SS-POST	10	10		25	23	21 (4.0)	15	15	19 (30)
	9.6			22			14		

[a] Concentrations rounded to two significant figures. [b] ST muscle = injection site.

Table 2. Pen G Muscle and Muscle Juice Levels:[a] Withdrawal Day 2

	cow 4			cow 5			cow 6		
	muscle		muscle juice	muscle		muscle juice	muscle		muscle juice
sample	ng/g	average ng/g	average ng/mL (% RSD)	ng/g	average ng/g	average ng/mL (% RSD)	ng/g	average ng/g	average ng/mL (% RSD)
HT-ANT	12	12	11 (40)	1.2	1.1	1.1 (4.1)	5.7	6.2	6.2 (11.0)
	13			0.9			6.8		
HT-POST	9.0	9.7	7.1 (9.2)	1.0	1.0	1.0 (16)	7.2	7.4	5.6 (6.3)
	10			1.0			7.6		
NK-ANT	8.2	7.3	7.7 (7.4)	1.4	1.4	1.7 (19)	5.7	5.9	5.2 (9.7)
	6.4			1.4			6.1		
NK-POST	6.2	6.6	6.4 (12)	1.5	1.5	2.0 (19)	17	17	11 (42)
	7.0			1.6			17		
TG-TIP	8.8	8.6	9.6 (11)	1.8	1.8	1.8 (6.6)	7.7	7.7	7.6 (14)
	8.4			1.8			7.7		
ST-ANT[b]	16000	18000	37000 (160)	7.7	7.5	8.6 (62)	13000	11000	44000 (68)
	21000			7.2			9100		
ST-POST[b]	1300000	1200000	270000 (210)	75000	73000	530 (44)	440	460	540 (67)
	1000000			71000			470		
SM-DOR	18	18	14 (25)	4.0	4.2	6.9 (93)	13	13	10 (63)
	19			4.4			13		
SM-LAT	340	330	330 (180)	1.3	1.5	4.1 (80)	9.3	8.9	11 (90)
	320			1.6			8.4		
VL-DOR	7.0	7.3	6.5 (13)	0.6	0.6	0.9 (16)	12	14	23 (110)
	7.6			0.6			15		
VL-LAT	8.6	8.2	6.6 (19)	0.5	0.6	1.0 (15)	82	85	30 (50)
	7.8			0.7			89		
FS-ANT	7.4	8.4	5.6 (20)	1.0	0.9	1.0 (30)	4.9	4.6	6.3 (13)
	9.4			0.9			4.4		
FS-POST	7.2	7.0	6.0 (28)	0.9	0.9	0.8 (19)	6.8	6.6	5.0 (19)
	6.7			0.9			6.4		
LD-ANT	7.0	6.6	6.6 (18)	0.7	0.8	0.6 (11)	4.1	4.1	5.7 (16)
	6.3			0.8			4.1		
LD-POST	10	9.5	8.4 (30)	0.8	0.8	0.9 (20)	3.4	3.6	3.0 (17)
	8.9			0.8			3.7		
SS-ANT	6.6	6.4	5.1 (4.4)	1.2	1.2	1.0 (13)	6.0	6.1	5.0 (13)
	6.3			1.2			6.2		
SS-POST	5.3	4.8	4.5 (9.8)	1.0	0.9	0.8 (14)	6.7	6.5	4.6 (16)
	4.3			0.9			6.4		

[a] Concentrations rounded to two significant figures or one near the reporting limit. [b] ST muscle = injection site.

Table 3. Pen G Liver and Kidney Levels[a]

		liver			kidney	
WDR day	cow	ng/g	average (% RSD)	*n*	average (% RSD)	*n*
1	1		2100 (2.6)	3	1500 (5.9)	3
1	2	L1−2200, 2500 L2−1800, 1800 L3−1800, 1700 L4−1900, 1800 L5−2000, 1700 L6−1800, 2000 L7−1800, 1700 L8−1800, 1700	1900 (12)		2200 (1.6)	3
1	7		670 (1.4)	3	340 (7.6)	3
2	4		900 (1.3)	3	550 (5.1)	3
2	5		170 (7.8)	3	140 (4.8)	3
2	6		600 (4.9)	3	360 (2.1)	3

[a] Concentrations rounded to two significant figures.

The previous study with tilmicosin also found considerable variation among subsamples (3.3 g each) of a given muscle (*5*). It is interesting to note that the differences seen within muscle and by others with kidney (*1*,*2*) may not be present to the same extent in liver. In the case of cow 2, 8×100 g portions sampled from a variety of locations within the liver (L1−L8) were homogenized and then analyzed as two replicates each. Less variation is observed [16% relative standard deviation (RSD)] between these samples, suggesting that that liver may be a more homogeneous matrix.

Comparison of Pen G Residues in Muscle Juice and Muscle. Extraction of residues from muscle is typically more difficult than extraction from a liquid matrix, such as serum, because of the mechanical grinding that is required to ensure efficient extraction of muscle, as opposed to simple vortex mixing that can be sufficient for liquid matrices. Thus, we sought to determine whether muscle juice, produced from muscle by squeezing in a vise, would provide an effective alternative route for the determination of Pen G residues in muscle. Juice was readily produced using this approach, yielding an average of approximately 5 mL of juice/20 g of muscle and a ~2−9 mL range produced between all muscles. For a given cow/muscle, volumes of juice obtained were typically within 1−2 mL of the average for that cow/muscle. **Tables 1** and **2** compare the Pen G levels found in muscle juice versus muscle. In some cases, the average values for the two matrices show very good agreement, while in other cases, they do not, with the values for muscle juice showing large percent RSDs.

Table 4. Comparison of Pen G Levels in Muscle, Muscle Juice, and Homogenized Muscle Juice

	muscle (homogenized)		muscle juice	juice from homogenized muscle	
muscle sample[a]	ng/g	average ng/g	average ng/mL (% RSD)	ng/mL	average ng/mL
HT-ANT	46			55	
	48	47	88 (76)	52	54
HT-POST	27			34	
	29	28		33	33
VL-DOR	26			24	
	24	25	25 (6.2)	25	24
VL-LAT	38			37	
	39	38	44 (24)	40	38
TB-ANT	27			26	
	24	26	29 (12)	23	24
TB-POST	18			16	
	16	17	19 (8.2)	16	16

[a] Cow 2.

This is probably a reflection of the variation in the muscle, coupled with the smaller subsamples (20 g) taken for muscle juice sample production. High percent RSDs obtained for Pen G values in some muscle juices are not necessarily linked to the juice volume obtained. For example, juice samples obtained for LD-ANT, cow 7, were all ~3 mL volume; however, the Pen G levels among these samples displayed a 70% RSD. On average, however, we obtained a juice versus muscle Pen G concentration ratio of 1.06 when considering all results obtained for day 1 and 2 of withdrawal, except for juice/muscle results from muscles located close to the injection site. This shows an overall good correlation between juice and muscle levels, indicating that muscle juice could be a suitable matrix for screening purposes. This is true even for the muscles located close to the injection site, for which the juice results show larger variability but still clearly indicate that the muscle levels significantly exceed the tolerance. Thus, the use of small muscle samples to produce juice samples for analysis, such as is currently performed in the Premi® Test, may give acceptable results for screening purposes. If quantitation is desired, it may be necessary to either produce juice from a larger, more representative sample or to take an average of multiple smaller samples.

While this issue will require further study, it is likely that taking comparably sized samples of muscle to determine muscle and juice levels would provide better agreement for quantitative purposes. For example, several homogenized muscle samples (100 g) from cow 2, which had been used for muscle Pen G analyses, were squeezed to produce homogenized muscle juice samples. In these cases, the value for Pen G in the homogenized muscle juice was in good agreement with the value in homogenized muscle (**Table 4**). This is particularly clear for HT-ANT, for which the homogenized muscle juice results were much closer to those of homogenized muscle than the juice directly produced from the smaller subsamples.

In conclusion, this study indicates that Pen G residue levels can vary between and even within individual muscles. These variations for the most part follow no predictable pattern among different cows or withdrawal times, and the potential for such variation should be considered when sampling for monitoring purposes. With regard to Pen G in cattle, it may be reasonable to focus sampling for monitoring purposes on liver or homogenized kidney, to obtain more consistent results; however, there may be instances for which knowledge of Pen G (or other antibiotic) levels in muscle is required. Further study of this phenomenon could lead to the establishment of a standard muscle sampling protocol, in which one type of muscle is specified for sampling purposes, to promote sampling consistency. Given the potential variation among different muscles, it would be advisible for researchers to report the specific muscle(s) that they are using in their studies rather than simply reporting their matrix as "muscle". With regard to the potential for variation within a given muscle, small sample sizes should be avoided wherever possible, sampling ≥100 g portions of a given muscle. Finally, while the analysis of muscle juice as a replacement for muscle needs further study, it still seems promising for screening purposes. For quantitative purposes, juice would need to be produced from a large enough portion of a muscle to be representative or an average value of Pen G juice levels from several smaller portions would be needed.

ACKNOWLEDGMENT

Jonathan Leith, Jim Piatt, and Dale Buschling are acknowledged for assistance in obtaining incurred tissue samples, and Limei Yun and Alan Lightfield are acknowledged for technical assistance in sample analysis.

LITERATURE CITED

(1) Meenagh, S. A.; McEvoy, J. D. G.; Elliott, C. T. Determination of carazolol residues in porcine tissue by radioreceptor assay. *Anal. Chim. Acta* **2002**, *462*, 149–156.
(2) Cooper, A. D.; Tarbin, J. A.; Farrington, W. H. H.; Shearer, G. Aspects of extraction, spiking and distribution in the determination of incurred residues of chloramphenicol in animal tissues. *Food Addit. Contam.* **1998**, *15*, 637–644.
(3) Mortier, L.; Daeseleire, E.; Huyghebaert, G.; Grijspeerdt, K.; Van Peteghem, C. J. Detection of residues of the coccidiostat diclazuril in poultry tissues by liquid chromatography−tandem mass spectrometry after withdrawal of medicated feed. *J. Agric. Food Chem.* **2005**, *53*, 905–911.
(4) Reyes-Herrera, I.; Schneider, M. J.; Cole, K.; Farnell, M. B.; Blore, P. J.; Donoghue, D. J. Concentrations of antibiotic residues vary between different edible muscle tissues in poultry. *J. Food Prot.* **2005**, *68*, 2217–2219.
(5) Beechinor, J. G.; Bloomfield, F. J. Variability in residue concentrations of tilmicosin in cattle muscle. *Vet. Rec.* **2001**, *149*, 182–183.
(6) Mastovska, K.; Lightfield, A. R. Streamlining methodology for the multiresidue analysis of β-lactam antibiotics in bovine kidney using liquid chromatography−tandem mass spectrometry. *J. Chromatogr., A* **2008**, *1202*, 118–123.
(7) Code of Federal Regulations 2009, Title 21, Section 522.1696b.
(8) http://www.merckvetmanual.com, Table 30: Drug Withdrawal and Milk Discard Times of Penicillins.
(9) http://www.aoac.org/testkits/testedmethods.html.

Received for review October 9, 2009. Accepted March 31, 2010. The mention of trade names or commercial products in this paper is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.