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Potential of phytoceuticals to affect antibiotic residue detection tests in cow milk in a randomised trial

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ABSTRACT

Mastitis is a costly disease for dairy farmers. Some dairy farmers use herbal products, or phytoceuticals, to treat mastitis. Phytoceuticals have not been approved for this use by the United States Food and Drug Administration, and have not been tested to determine how they impact antibiotic residue detection testing. The current study tested the potential for phytoceuticals to cause positive results on two milk antibiotic residue screening tests, the Delvotest P and Charm SL Beta-lactam test, or to interfere with the detection of antibiotics by these tests.

The three phytoceuticals tested were labelled for intramammary, topical or intravulvar administration. Testing was performed in vitro using the products diluted in milk obtained from healthy organic dairy cows. Phytoceuticals were tested at concentrations ranging from 1.5 per cent to 100 per cent. Concentration levels were replicated at least twice on each milk antibiotic residue screening test. The Delvotest P is based on detection of bacterial inhibitors and no positive results were obtained for any product at concentrations less than 50 per cent. The Charm SL Beta-lactam test uses a receptor for the detection of beta-lactam antibiotics and no concentration of phytoceuticals caused an interference with these tests. Based on dilution of the products in bovine milk at physiologically achievable levels, phytoceutical products tested at levels expected after treatment do not cause positive test results for the Delvotest P nor do they interfere with the Charm SL Beta-lactam test in detection of various antibiotics.

INTRODUCTION

Mastitis is a common and costly disease for dairy cow farmers. Because mastitis is typically caused by bacterial infections, antibiotics are often used for mastitis therapy in conventional herds. Some dairy farmers augment antibiotic therapy with herbal products (hereafter referred to as phytoceuticals) such as topical mint udder creams or liniments. Organic farmers in the USA are not allowed to use synthetic antibiotics, except in cases where alternatives fail. If a USDA certified organic cow in the USA is administered synthetic antibiotics, she permanently loses her organic status (USDA 2013). Some organic dairy farmers in the USA reportedly manage mastitis using vitamin supplementation, whey-based products and phytoceuticals (Stiglbauer and others 2013, Pol and Ruegg 2007, Mullen and others 2013). The meat and milk withholding periods for these products as well as those phytoceuticals used by conventional farmers have not been determined in dairy cattle, and these products have not been approved by the United States Food and Drug Administration (US FDA). Withholding time has been estimated for one intramammary phytoceutical investigated in the current study, in goats (McPhee and others 2011), but there are no withholding data from cows for any of the products included in this study. Milk is regularly tested prior to processing to ensure that the milk has not been adulterated and that it meets quality standards for consumption. Adulteration is evaluated by testing for pesticide and drug residues using 'test methods that are validated by the FDA and accepted by the National Conference on Interstate Milk Shippers' (US Department of Health and Human Services 2009). To date, there are no test methods for detecting phytoceutical residues. The current study was designed to examine two industry standard tests to determine the tests' responses to phytoceutical presence in commingled cow milk and to determine if phytoceuticals would interfere with detection of antibiotics by the tests. It would be beneficial to have methods to detect these phytoceuticals to ensure that milk from cows dosed with these products is free of residues.

Some phytoceuticals and phytoceutical components have shown antibacterial activity in vitro for mastitis pathogens (Ananda Baskaran and others 2009, Mullen and others 2014a, Mason and others 2015), but the limited data published on in vivo usage (Mullen and others 2014b) does not indicate consistent antibacterial activity for the intramammary (IMM) product (Phyto-Mast,

CowMaster LLC, Narvon, PA, USA) included in this study. The main antibacterial ingredient in Phyto-Mast, thyme essential oil, has antibacterial activity in vitro on mastitis pathogens (Mullen and others 2014a). Other phytoceutical products available in the USA include a garlic-based tincture administered via the oral or intravulvar route (Dr. Paul's CEG Tincture, Arcadia, WI, USA) and an oregano-based topical product (Uddersol, Ralco, Marshall, MN, USA). Oregano contains carvacrol and thymol, potent antimicrobial molecules also present in thyme (Helander and others 1998). Garlic also has antibacterial activity in vitro (Cowan 1999). The effectiveness of these specific topical (TOP) and intravulvar (IVU) products for mastitis control has not been scientifically evaluated. However, a topical mint-based liniment treatment was evaluated by Knight and others (2000), who found that use of the topical liniment was not associated with any significant reduction in bacterial numbers compared with control following intramammary challenge with staphylococci. While in vitro activity has been demonstrated for compounds in thyme, garlic and oregano, it must be stressed that no efficacy data are available for these compounds after administration to dairy cows and effects of dilution and metabolism in the mammary gland are unknown.

Because phytoceuticals may have antibacterial properties, their ability to produce positive readings by milk antibiotic residue screening tests needs to be determined. Detection would result in failure to meet regulatory standards, potentially compromise food safety and decrease consumer confidence in dairy products. Based on the mechanism of the test, bacterial inhibition by components of the phytoceuticals feasibly could produce positives using the Delvotest P. For the Charm ROSA test, the concern would be interference from phytoceuticals because of the mechanism of the test. The Charm ROSA test uses bacterial receptors in a lateral flow design (Salter and others 2001). The receptors have affinity for beta-lactam drugs and presence of a beta-lactam influences the test strip visual appearance when flowing receptors bound to particles collect at either a control or test line on the test strip (Salter and others 2001). Stereochemical interference from the phytoceutical could influence the test's mechanism, leading to erroneous results.

The US FDA does not rigorously regulate herbs and supplements used in humans or in animals (Whole Foods 2017). Strength, purity and safety of these products cannot be guaranteed and, for a variety of reasons, the effects observed with use may vary (Whole Foods 2017). Based on the present state of regulatory practices and the limited data on safety and efficacy, there is the potential for side effects from pharmacologically active components, contaminants or drug interactions (Bent 2008). Factors such as lack of standardisation and variation among manufacturers and lots contribute to making it difficult to give precise information on toxicity. For thyme, there are no well-documented clinical data; however, traditional health practice patterns, the opinions of experts and anecdotal evidence suggest that thyme is tolerated well at doses commonly used (Basch and others 2004). The majority of adverse events reported include dermatological or allergic reactions (Basch and others 2004). Essential oil of thyme is not to be used by the oral route due to reported toxic events such as nausea and respiratory arrest (Basch and others 2004). No specific common safety risks are associated with use of oregano oil products (Kinder et al., 2015). However, allergies, blood pressure effects, difficulty breathing and speaking, itching and swelling (eg, eyelids, face, tongue) have all been reported after oregano intake (Whole Foods 2017). It has been reported that oregano used at medicinal doses 'may cause abortion, allergic skin reactions (bacterial skin infection and tenderness), central nervous system (CNS) depression and changes in mineral absorption (copper, iron and zinc)' (Whole Foods 2017). Garlic has been reported to cause mild gastrointestinal effects and there are case reports of bleeding (Bent 2008). It is noted that essential oils of herbs can be toxic when taken even in relatively small quantities (Kinder and others 2015). However, typical daily doses of oregano essential oil for humans contain between 165 and 195 mg of carvacrol (Kinder and others 2015). Our research group has reported that, after receiving topical oregano oil, the highest concentration of carvacrol detectable in plasma is 0.003 µg/ml (Mason and others 2017), which would require a human to consume 5,500 litres of blood in order to meet the normal daily dose of carvacrol.

No residue level of phytoceuticals is acceptable in milk because these products have not been approved by the US FDA (US Food and Administration 1994). Because of their widespread use and different mechanisms of actions, two milk antibiotic residue screening tests were used: the Charm SL Beta-Lactam test (SLBL, ROSA Pearl Reader, Charm Sciences, Lawrence, MA, USA) and the Delvotest P (DSM, Delft, The Netherlands). Charm SLBL accounted for the largest number of tests used in the USA for residue testing on milk from October 2013 to September 2014 and Delvotest P was the third most frequently used test during this time (GLH Incorporated 2014). The mechanism of each test differs: Charm SLBL uses receptor protein-antibiotic binding specific for beta-lactams, and Delvotest P uses microbial inhibition. Those tests were chosen because they are commonly used, have two different mechanisms, are from two different manufacturers and detect a broad range of antimicrobial activity (Delvotest P).

The purposes of this study were to determine how selected antibiotic residue screening tests (Delvotest P or Charm SLBL) would be affected when various concentrations of three phytoceuticals were diluted in bovine milk and to determine if a phytoceutical could interfere with antibiotic detection by Charm tests. We hypothesised that we may get positive test results from the inhibitory effects of the phytoceuticals on the Delvotest P and that the concern for the Charm SLBL test would be potential

interference from phytoceutical based on the test's mechanism.

MATERIALS AND METHODS

Phytoceutical testing trials

Products tested

Three products marketed in the USA were tested. These included an IMM phytoceutical product (Phyto-Mast, CowMaster LLC, Narvon, PA), a TOP phytoceutical product (Uddersol, Ralco Animal Health, Marshall, MN, USA), and an intravulvar (IVU) herbal product (Dr. Paul's CEG Tincture, Arcadia, WI, USA). The purported active antimicrobial ingredients are thymol for the IMM, carvacrol for the TOP and diallyl disulfide for the IVU products. This pilot study used replicates of each concentration as described hereafter to determine the variability among replicates.

Milk sampling

Milk used in the antibiotic residue screening tests was commingled milk from 10 healthy USDA certified organic lactating cows. The cows were mid-lactation Holstein, Holstein cross or Jersey cows between their first and tenth lactations. All cows had four functional udder quarters and no visible signs of mastitis. Milk from all cows had composite SCC ≤146 000 cells/ml (mean: 27 900 cells/ml for each cow) as assessed by DeLaval DCC (DeLaval, Tumba, Sweden) and exhibited no growth on microbiological culture of aseptically collected quarter milk samples. Daily milk production per cow ranged between 11 and 40 kg. Milk was refrigerated on collection and used within 72 hour of milking.

Trial 1. Products alone

In trial 1, the IMM, TOP and IVU products were tested at concentrations from 1.5 per cent to 5 per cent vol/vol in 0.5 per cent increments for three replicates per concentration and at 10 per cent, 25 per cent, 50 per cent and 100 per cent for two replicates per concentration, diluted in the raw bulk tank milk (Tables 1 and 2). The lower concentrations were expected to encompass physiologically achievable concentrations after use, and the higher concentrations were tested later with another batch of milk from a different group of 10 cows with the same qualifications as described under'Milk Sampling'. Each concentration was tested using two antibiotic residue screening tests: Charm SLBL and Delvotest P (Tables 1 and 2). Each concentration/product/test combination was randomised by date and technician using the random number generator of random.org, blocking by antibiotic residue detection test and treatment runs were assigned by KAEM using the random numbers generated. All tests were run in the Center for Chemical Toxicology Research and Pharmacokinetics in the College of Veterinary Medicine at North Carolina State University, Raleigh, North Carolina, USA.

TABLE 1: Delvotest P results from testing various concentrations of three different phytoceutical products in raw milk from organic cows

Concentrations (per cent)	# positive/# replicates tested		
	IMM	TOP	IVU
1.5	0/3	0/3	0/3
2.0	0/3	0/3	0/3
2.5	0/3	0/3	0/3
3.0	0/3	0/3	0/3
3.5	0/3	0/3	0/3
4.0	0/3	0/3	0/3
4.5	0/3	0/3	0/3
5.0	0/3	0/3	0/3
10	0/2	0/2	0/2
25	0/2	0/2	0/2
50	2/2	0/2	0/2
100	2/2	2/2	0/2

IMM, intramammary herbal product;
TOP, topical herbal product;
IVU, intravulvar herbal product.

Delvotest P procedures

Penicillin-positive control was prepared by adding 15 ml deionised water to the penicillin included in the test kit

TABLE 2: Charm SL Beta-lactam results for phytoceutical products in raw milk

Concentrations (per cent)	Reader results. # positive/# tested (# invalid) indicated		
	IMM	TOP	IVU
1.5	1/3	0/3	0/3
2.0	0/3	0/3	0/3
2.5	0/3	0/3	0/3
3.0	0/3	0/3	0/3
3.5	0/3	0/3	0/3
4.0	0/3	0/3	0/3
4.5	0/3	0/3	0/3
5.0	0/3	0/3	0/3
10	1/2	0/2	0/2
25	0/2	0/2	0/2 (2 invalid)
50	2/2	0/2 (1 invalid)	0/2 (2 invalid)
100	0/2 (2 invalid)	0/2 (2 invalid)	0/2 (2 invalid)

Results are given as printed by the ROSA Pearl Reader. Any value 0 or lower is considered a negative result and any value greater than 0 is considered a positive result. 'Invalid' indicates that the test strip was not valid, most likely due to high concentrations of the phytoceutical interfering with flow in the test strip.
IMM, intramammary herbal product;
TOP, topical herbal product;
IVU, intravulvar herbal product.

to create a solution of 5 ppb penicillin, which was mixed by inversion until a uniform solution was reached and allowed to sit 30 minutes to equilibrate to room temperature. The control was remixed before using. Spiked milk samples were prepared for each phytoceutical product and for the positive control using unadulterated raw milk in 1.5 ml centrifuge vials. Vials were vortexed for 10 seconds immediately after spiking the milk. The Delvotest P ampules were prepared according to manufacturer directions: a nutrient tablet was placed into each ampule, then 100 µl of sample was pipetted into each individual ampule. Each incubator run had a positive control ampule (prepared using the 5 ppb penicillin solution) and a negative control ampule (antibiotic-negative raw milk) as well as the experimental samples. The ampules were placed into preheated incubators. After the ampules were in the incubator for 2.5 hours at 64°C per manufacturer recommendations, the ampules were removed, and the color of the agar was inspected. Purple agar indicated a positive result, and yellow agar indicated a negative result. If there are no antibiotics in the milk, the bacteria *Geobacillus stearothermophilus* var. *calidolactus* grows in the incubated ampule and undergoes lactic acid fermentation, which decreases the pH, thus turning the agar yellow. If there are inhibitors in the milk, the bacterial growth is inhibited, and the agar remains purple.

Charm SLBL procedures

The spiked milk samples were prepared for each phytoceutical product with antibiotic-negative raw milk. Each sample tube was vortexed for 10 seconds immediately after spiking the milk and again before the 300 µl sample was pipetted into the test strip well, which had been equilibrated to room temperature. The test strip was immediately resealed. Test strips were placed in the quad incubator for 8 min and then read on the calibrated reader (ROSA Pearl Reader, Charm Sciences). As per the manufacturer's instructions, the control line (denoted by C) must be complete to be a valid test. If the control line was absent, partial or indistinct, the test was considered 'invalid' and was repeated. Additionally, the positive control test strip had to read +400 or more positive, and the negative control test strip had to read −600 or more negative to have valid tests.

Statistical analysis

Binomial models were constructed using PROC GLIMMIX of SAS V.9.3 (SAS Institute, Cary, NC, USA) to assess the randomisation and determine the odds of positive results at each of the concentrations tested.

Trial 2. Potential for phytoceutical product to interfere with various Charm tests

Charm ROSA tests are commonly used to determine the presence or absence of various antibiotics in milk. In trial 2, a two-part process was used to evaluate the ability of the IMM product to cause positive Charm test results using the Charm ROSA SLBL test (part 1; Table 3) or to interfere with detection of penicillin and other antibiotics using other Charm test formats (part 2; Table 4). For part 1, with 4 replicates, the IMM product was diluted in antibiotic-free raw milk to produce concentrations of the IMM product at 1, 2.5, 5, 10 and 20 per cent solutions. Additionally, negative (antibiotic-free milk) and positive (5 ppb penicillin G control mixed in a 2.5 per cent solution of IMM) controls were included. Part 2 was designed to determine the ability of the IMM product at 2.5 per cent to interfere with testing by various Charm ROSA test formats. Trial 2 was run at Charm Sciences.

TABLE 3: Results obtained from testing interference of various concentrations of IMM on Charm SL Beta-Lactam (ROSA SLBL) test

Sample ID	SLBL test results, interpretation of four replicates
Negative control, raw milk	4/4 negative
Positive control (5 ppb penicillin G in 2.5 per cent IMM solution)	4/4 positive
20 per cent solution IMM	4/4 negative
10 per cent solution IMM	4/4 negative
5 per cent solution IMM	4/4 negative
2.5 per cent solution IMM	4/4 negative
1 per cent solution IMM	4/4 negative

Positive result: the test indicates that the antibiotic is present.
Negative result: the test indicates that no antibiotic is present.
IMM, intramammary phytoceutical product.

All tests used were Charm ROSA tests, including Charm SLBL (US safe level for beta-lactams (BL)), SL3 (safe level for BL), Sulfa Drug Test, MRLBL (test for BL at maximum residue level (MRL)), MRLTET (test for tetracyclines at MRL), MRLBLTET (test for tetracyclines and BL at MRL) and chloramphenicol. The ROSA Pearl reader (Charm Sciences) was calibrated according to the manufacturer's directions before each round of testing (at least once daily), and the ROSA quad incubator (Charm Sciences) was preheated to 56°C (±1°C). Negative controls were included in each run and were prepared by adding 1 per cent and 2.5 per cent IMM in antibiotic-free milk. Positive controls were prepared by mixing the antibiotic detected by the test in the antibiotic-free milk containing 2.5 per cent IMM (Table 4).

RESULTS

Trial 1. Phytoceutical products alone

Statistical analysis revealed that date and replication number had no effect on results obtained and that the statistical odds of a positive outcome on each of the tests at each concentration level were the same as the reported results.

TABLE 4: Trial 2: Results of testing various Charm tests in the presence of positive controls and 2.5 per cent solutions of the IMM product, as well as 1 per cent and 2.5 per cent solutions of the IMM product alone

	Charm SL3 test	Charm sulfa test	Charm MRLBL test	Charm MRLTET/MRBLTET tests	Charm CAP test
Negative control (1 per cent IMM)	Negative	Negative	Negative	Negative	Negative
Negative control (2.5 per cent IMM)	Negative	Negative	Negative	Negative	Negative
Positive control					
2.5 per cent IMM with:					
5 ppb penicillin G	Positive	N/A	Positive	N/A	N/A
10 ppb sulfa-methazine	N/A	Positive	N/A	N/A	N/A
5 ppb penicillin G+100 ppb oxytetracycline	N/A	N/A	Positive	Positive	N/A
0.5 ppb chloramphenicol	N/A	N/A	N/A	N/A	Positive

Positive result: the test indicates that the antibiotic is present.
Negative result: the test indicates that no antibiotic is present.
N/A: the antibiotic listed in the left column is not detectable by the Charm test listed and thus was not tested.
CAP, chloramphenicol; IMM, intramammary phytoceutical product.

Delvotest P

Negative Delvotest P results were obtained for all concentrations of each of the three phytoceutical products at or below 5 per cent concentration (Table 1); all replicates of 10 per cent and 25 per cent were also negative. Concentrations that tested positive included both replicates of 50 per cent and 100 per cent IMM as well as both replicates of 100 per cent TOP (Table 1). All results were negative for IVU and the remaining concentrations of IMM and TOP.

Charm SLBL

Negative Charm SLBL results were found for all but one test for concentrations up to and including 5 per cent of each of the products (Table 2). We observed positive results for one of three replicates of 1.5 per cent IMM. We observed positive results for one of two replicates of 10 per cent IMM. Positive results were also obtained for both replicates of 50 per cent IMM. 'Invalid' results were obtained for both replicates of 100 per cent for all three products (see Table 2). Additionally, 'invalid' tests were also seen for both IVU replicates at 50 per cent, and one replicate of 50 per cent TOP.

Trial 2. Effect of intramammary phytoceutical product at various concentrations on Charm test

Influence of IMM product on SLBL test

None of the concentrations of the IMM product produced positive results for SLBL test, as shown in Table 3. The 5 ppb penicillin G-positive control with 2.5 per cent IMM product added produced a positive test result, indicating that the 2.5 per cent IMM product did not interfere with detection of penicillin by the test. Negative control milk, as well as milk samples with 1 per cent, 2.5 per cent, 5 per cent, 10 per cent and 20 per cent IMM, produced negative results on the SLBL test.

Testing various Charm tests in the presence of positive controls and 2.5 per cent solutions of the IMM product, as well as 1 per cent and 2.5 per cent solutions of the IMM product alone

The potential interference of 2.5 per cent IMM product was tested using five additional Charm test formats. Concentrations of IMM at 1 per cent and 2.5 per cent in antibiotic-free milk were negative for all test formats (Table 4). When the positive antibiotic controls specific for each of the five Charm test formats were prepared with 2.5 per cent IMM, all tests were appropriately positive, indicating no interference by 2.5 per cent IMM (Table 4).

DISCUSSION

The goals of this research were to determine the potential effects of three phytoceutical products on two commonly used antibiotic residue screening tests and to determine if phytoceuticals can interfere with antibiotic detection tests. For the Delvotest P, the major concern was whether potential inhibitory effects of

the phytoceuticals would produce positive test results. For the Charm ROSA tests, the main concern would be interference by phytoceuticals on the test mechanism. These concentrations were chosen to represent what could be physiologically achievable in dosed cattle as well as much higher concentrations to determine if the products were able to cause positive readings on these tests. If we assume that the average organic cow produces 19.5 kg/day of milk (Stiglbauer and others 2013), administration of one 12 ml dose of IMM product in one-quarter would result in a concentration of 0.06 per cent vol/vol. Even if milk production was reduced, for example, by 80 per cent in a case of mastitis and the farmer dosed all four quarters, the concentration of IMM in milk would only reach 1.2 per cent. Concentrations achievable by the IVU and TOP products would be even lower, depending on the proportion of the absorbed dose that reached the udder. Additionally, the tests are intended for bulk tank or commingled milk and not individual cow milk; the concentration would be decreased when the individual cow milk is pooled in the tank. These products have not been examined postadministration to determine the extent to which they are metabolised before excretion in milk. However, these compounds can be detected in milk (Armorini and others 2016). Apart from one replicate of 1.5 per cent IMM with one test, no positive results were obtained with the residue detection tests for concentrations of all three products up to and including 5 per cent vol/vol. There were no positive results obtained from the IVU product, suggesting that either it has no detectable antibacterial activity or that the testing methods could not detect this product.

Both the mechanism of action of the detection tests and the chemical nature of the phytoceutical products would influence the test results. All three phytoceutical products contain semi-volatile substances. The bacteria *G stearothermophilus* var. *calidolactus* in the Delvotest P ampules are aerobic. During preparation of the spiked milk samples, capped tubes were used, but the ampules in the incubator require open tops to allow aerobic respiration for *G stearothermophilus* var. *calidolactus*. Because the ampules are in the incubator for 2.5 hours, the heat, time and air exposure may affect the amount of phytoceutical product retained within the ampule over time. The Delvotest P may have only detected the phytoceutical products at high concentrations due to their antibacterial components' semi-volatility. The Charm SLBL test strips had sealed chambers for the samples, thus inhibiting entry of air, which would decrease the volatilisation of antibacterial components of the tested phytoceuticals. However, there were not more positive Charm SLBL tests at lower concentrations than Delvotest P tests.

Although the ingredients of the phytoceuticals have not been fully characterised, components like thymol, with known antibacterial properties, can explain the positive results for the Delvotest P. The IMM product was expected to produce some positive results in the antibiotic residue screening tests because O'Donnell (2011) reported that IMM at 12.5 per cent concentration in milk produced a positive result on the Delvotest SP-NT. The current study, using Delvotest P, only had a positive result with IMM at 50 per cent or higher concentration. Because carvacrol is similar in structure to thymol, TOP was expected to produce some positive results in the milk antibiotic residue screening tests. There was no known previous study of IVU producing any positive results from antibiotic residue screening tests, so we had no basis to hypothesise whether positive tests might be obtained.

We hypothesised that the Delvotest P would be more likely to detect the presence of the phytoceuticals than the Charm SLBL because of its mechanism of action. Delvotest P uses microbial inhibition to detect antibiotic residues, whereas Charm SLBL is selective for antibiotics that contain a beta-lactam ring. For the Charm SLBL, the sample replicates agreed in results except for the 50 per cent vol/vol TOP samples. The 50 per cent vol/vol TOP samples included a negative result and an invalid result. These large differences in results between the same concentration samples may indicate an error in the test strip, reader or sampling. The inconsistent results from the Charm SLBL as well as the positive results obtained could be the result of flow problems within the testing apparatus: the IMM and TOP products are oil-based and high concentrations could impede flow within the testing device. However, the testing of interference of IMM on various Charm test formats reported negative results for up to 20 per cent IMM on the Charm SLBL, indicating reproducibility of the negative results.

Theoretically, the phytoceutical products could contain molecules other than thymol and carvacrol that may have beta-lactam rings on their structures or cross-react with the SLBL test to cause a positive reading on the Charm SLBL at sufficiently high concentrations. However, we have no evidence for the appearance of such structures in the phytoceuticals evaluated. Antibiotic residue screening tests for milk can also produce false-positive or false-negative results. Mastitic cows usually have high somatic cell count (SCC), and false-positive results are often associated with high SCC (Van Eenennaam and others 1993). However, high SCC (1 000 000/ml) have been reported not to interfere with the Charm SLBL (Salter and others 2001). Frequent occurrence of false-positive results can cause economic loss for the farmer because antibiotic-positive milk cannot be sold. One study found selectivity rates, where negative samples correctly identified to be negative by the test, were above 90 per cent for seven different antibiotic residue screening tests (Andrew and others 1997). One of these tests included Delvotest P, which was used by the current study. Other components that can cause false-positive results are lactoferrin, lysozyme, microbes and free fatty acids (Andrew and others 1997). In the current study, milk samples used

for testing had low SCC and thus any false positives were not likely caused by SCC.

Based on results of the phytoceutical products tested, use of those products in cows should not interfere with the tested antibiotic detection tests nor cause positive antibiotic residue detection tests by the Delvotest P at physiologically achievable concentrations in milk. None of these phytoceuticals interfered with the positive antibiotic controls of various Charm tests including Charm SLBL. It is important to note that any detectable residue of the phytoceutical products would be considered unacceptable, as the products have not gone through the FDA approval process for use as mastitis therapy. We conclude that these tests do not appear to interfere with these two antibiotic detection tests, even at levels above those achievable physiologically. Processors and farmers should not expect the tests evaluated here to detect phytoceuticals in bulk milk. A negative test result with either product would not eliminate the possibility of residues and further testing would be required for detection (Armorini and others 2016).

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