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Short communication: Determination of withdrawal time for oxytetracycline in different types of goats for milk consumption

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ABSTRACT

Antibiotics are widely used in animal husbandry and the presence of antibiotics in milk is a health hazard. The objective of this study was to determine residual amounts of oxytetracycline in fresh, aged, and pasteurized milk of 3 breeds of goats using HPLC analysis. It was also essential to determine the safe withdrawal period of oxytetracycline in lactating goats. The quantitative results obtained using the HPLC system were compared with the tolerance limit of oxytetracycline in milk in the United States. Fifteen milking does, 5 Nubians, 5 Alpines, and 5 LaManchas were randomly selected from the milking herd at the International Goat Research Center at Prairie View A&M University. A simple sample preparation and isocratic HPLC method using ultraviolet detection was used for analysis of milk samples. The HPLC results indicated that the withdrawal period of oxytetracycline in treated Alpine does was 82 h (7 milking), whereas for Nubian does the period was 58 h (5 milking), and for LaManchas the period was 72 h (6 milking) after drug administration. The overall withdrawal period for all the treated goats of 3 breeds was 72 h. Although these results indicated that the depletion rate of this antibiotic was faster in goats than the reported data for cows, the 96-h withdrawal period that is currently used for lactating cows is still necessary for these 3 breeds of goats. Additionally, our results indicated that oxytetracycline is not stable in goat milk at refrigeration temperature or during pasteurization and will decrease significantly.
Key words: oxytetracycline, residues, goat milk, withdrawal period

Short Communication

Milk has been an important food for humans since the domestication of dairy animals. It is a common component of the animal-derived food products that comprise many diets. Methods to ensure the safety and quality of goat milk and milk products are necessary with the expansion of goat dairy industry and use of goat milk in various sectors of food processing. Therefore, it is important to make sure that marketed goat milk is unadulterated and safe for human consumption.

Antibiotics are widely used in animal husbandry for the treatment of diseases, health maintenance, and, in some countries, at subtherapeutic levels as feed additives to suppress undesirable bacteria (Pharmacia and Upjohn, 1998; Kelly et al., 2004; Sawant et al., 2005). The improper use of antibiotics for the control of diseases such as mastitis is the major source of drug residues found in milk (Sawant et al., 2005; HHS, 2010). Concern exists that antibiotic residues in foods could significantly shift the resistance patterns in the microbial population in the human intestinal tract (Kelly et al., 2004; Jones 2009). The concerns about antibiotic resistance exist from food-borne pathogens such as *Salmonella*, *Campylobacter*, and enterococci (Pharmacia and Upjohn, 1998; HHS, 2010).

The presence of antibiotic residues in milk is known to interfere with the manufacture of several fermented dairy products by inhibiting starter activity, which can lead to monetary losses (Mayra-Makinen, 1995; Hays, 2003; Jones, 2009). Milk supplies containing antibiotics above certain concentrations are illegal. The maximum concentrations of residues are set for different antibiotics so that no unintended harmful effects are likely to occur from these drugs (Hays, 2003; FDA, 2013).

Tetracyclines are a group of broad-spectrum antibiotics that are active against gram-positive and gram-negative bacteria such as chlamydia, mycoplasmas, rickettsiae, and protozoan parasites. The tetracycline group is currently used to treat goats for diseases such as mastitis, pink eye, and urinary and enteric infections (Pharmacia and Upjohn, 1998). They are generally regarded as relatively nontoxic, but they could produce a large number of adverse effects, some of which can be life threatening under certain circumstances. Those effects include superinfection, diarrhea, ingestion, direct toxicity, irritation, dizziness, antianabolic effects, photosensitivity, and allergic symptoms in humans.

Current methods for detection of antibiotic residues in milk include tests such as microbiological inhibition

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tests, immunoassay tests, and chemical or physical methods such as spectrophotometric and chromatographic methods (Podhorniak et al., 1999; Sierra et al., 2009; Beltrán et al., 2013). The available microbiological tests are nonspecific for some antibiotics, such as tetracyclines and penicillins, whereas immunoassays are usually quite expensive. A quantitatively accurate chemical method for detecting antibiotics in milk is HPLC. Considerable progress has been reported in the development of HPLC methods for determination of tetracyclines and other antibiotics in both milk and meat tissues (Furusawa, 2003; Andersen et al., 2005; Fritz and Zuo, 2007; Fletouris and Papapanagiotou, 2008).

The Food and Drug Administration (**FDA**) established guidelines for the use of oxytetracycline (Liquamycin LA-200; Pfizer, Brazil) injections in lactating dairy cows in 1998; prior to that, oxytetracycline was only approved for beef animals (FDA, 1998). With this approval, oxytetracycline is labeled for administration to beef and dairy animals for treatment of pneumonia, shipping fever complexes associated with *Pasteurella* spp., *Haemophilus* spp., pink eye, foot rot, bacterial enteritis, leptospirosis, wound infections, and acute metritis. The FDA and dairy industry set a new tolerance level of 300 ng/mL for tetracyclines in milk when the approval notice for oxytetracycline was published in the FDA (2013). In the Codex Alimentarius Commission (2009), the European Union, and other regulatory organizations in some countries, the maximum residue level for oxytetracycline in milk is 100 ng/mL (Veterinary Drug MRL Database; www.mrldatabase.com).

The withdrawal period is the waiting time that must elapse before treated animals or their products can be processed for human consumption (Pharmacia and Upjohn, 1998). The withdrawal period for this antibiotic for lactating cows is 96 h after the last treatment. However, the withdrawal period for this antibiotic has not been determined for milking goats. Goat milk producers currently use the withdrawal time recommended for lactating cows. Currently, considerable variation exists in the management practices associated with antibiotic use on dairy farms (Sawant et al., 2005). Goats are considered minor species and no FDA-accepted screening test exists for tetracyclines in goat milk (FDA, 1998). It is important to quantitatively determine the exact residual amounts of these antibiotics in goat milk and to calculate the safe withdrawal period of treated lactating goats. Information on the safe withdrawal periods for antibiotic residues in goat milk is limited and to our knowledge the depletion rate of oxytetracycline in the Alpine, Nubian, and LaMancha breeds of goats has not been previously determined. Therefore, the objectives of our study were (1) to determine the residual amounts of oxytetracycline in the milks of Alpine, Nubian, and LaMancha goats for a period of time with a quantitative technique (HPLC), (2) to determine the safe withdrawal periods of oxytetracycline residues in milk of Alpine, Nubian, and LaMancha breeds of goats treated intramuscularly with this antibiotic, and (3) to determine the levels of oxytetracycline residues in goat milk treated differently, such as fresh, 72-h raw milk, and pasteurized milk.

Animal Selection and Treatment

Fifteen milking does, 5 Nubian, 5 Alpine, and 5 LaMancha, were randomly selected from the herd at the International Goat Research Center at Prairie View A&M University. The selected does were average milk producers with BW ranging from 55 to 75 kg and were in mid lactation. Each goat was intramuscularly injected with oxytetracycline (Liquamycin LA-200; Pfizer Inc., New York, NY) in the hind leg according to animal BW. A dose of 8 mg of oxytetracycline per pound of BW was applied for all injections (1 mL/11.4 kg of BW) according to the manufacturer's recommendation. One milliliter of oxytetracycline contained 200 mg of amphoteric oxytetracycline base in the aqueous solution (manufacturer's certified concentration). Each doe was given 2 antibiotic injections, 1 in the evening before milking and the second injection 48 h later according to the recommended therapeutic practice.

Sample Collection

The treated goats were hand milked and a sample from each goat was placed on ice in a container and brought to the laboratory for analysis. Milk samples were collected twice daily in the mornings and evenings after the second antibiotic injection for up to 138 h. Each milk sample was divided into 3 portions. One portion of raw milk sample was aged in the refrigerator (at 4°C) for 72 h before analysis, whereas the second portion of sample was pasteurized (at 63°C for 30 min) and frozen until analysis. The third portion of the sample was analyzed the day they were collected. All fresh milk samples from treated animals were analyzed for oxytetracycline residues until depletion using the HPLC system.

Sample Extraction

Five milliliters of milk were deproteinized by mixing with 1 mL of 1 *M* HCl and then 15 mL of acetonitrile was added to the mixture according to the method of Moats and Harik-Khan (1995) that was modified. The modifications included filtering the supernatant (filter