J. Dairy Sci. 101:1–5
https://doi.org/10.3168/jds.2017-12991

Short communication: Effects of fluoxetine on lactation at weaning in sheep

P. L. Harrelson,*[1] D. M. Hallford,† and T. T. Ross†
*Department of Agricultural Sciences, Morehead State University, Morehead, KY 40351
†Department of Animal and Range Science, New Mexico State University, Las Cruces 88003

ABSTRACT

Selective serotonin reuptake inhibitors have been considered for use in the dairy industry to aid in dry-off procedures because of their ability to delay the onset of lactation. Fluoxetine (a selective serotonin reuptake inhibitor; FLX) is an agent that has been shown to delay the onset of lactogenesis stage II when taken during pregnancy and lactation in women. Two experiments were conducted to determine whether ewes would be an appropriate model to evaluate the effects of FLX on milk production at weaning. In the first experiment, 12 Suffolk cross ewes (body weight = 83.4 ± 12.2 kg; body condition score = 2.1 ± 0.4) in late lactation were assigned to treatments of 0 (control), 40, or 80 mg of FLX. They were given a single subcutaneous injection with the appropriate level of FLX mixed with propylene glycol at 0700 h on approximately d 78 of lactation (the day lambs were removed). In the second experiment, 18 Suffolk cross ewes (body condition score = 1.8 ± 0.3) from a previous lactation study were selected in late lactation. On approximately d 66 following parturition, weaning was initiated and ewes received a single oral bolus treatment (0, 80, or 160 mg of FLX). Treatment was administered using gelatin capsules containing the appropriate dose of FLX. For both experiments, milk production was estimated: in experiment 1 on d 0 (before treatment), 1, 2, and 3 (after treatment) at 0800 and 1100 h, and in experiment 2 on d 0, 1, and 2 following treatment at 0800 or 1100 h. Milk production was measured over a 3-h period. We observed no treatment differences or day effects on milk production in either experiment. In experiments 1 and 2, as the dose of FLX increased, milk production decreased linearly. Serum lactose concentrations were depressed in ewes treated with FLX in experiment 1 but similar across treatments in experiment 2. Overall, FLX depressed milk production in ewes; therefore, there is potential to use FLX as a dry-off agent in the dairy industry.

Received April 5, 2017.
Accepted August 31, 2017.
[1]Corresponding author: p.harrelson@moreheadstate.edu

Key words: dry-off, fluoxetine, lactation, sheep

Short Communication

Serotonin (**5-HT**) is found in mammary epithelial cells and milk. Stull et al. (2007) suggested that 5-HT is secreted through the basolateral membranes into the interstitium or it leaks out of the milk space where it is detected by 5-HT receptors. This leakage could occur through the tight junctions between mammary epithelial cells and could explain 5-HT feedback inhibition on lactation (Stull et al., 2007). Serotonin has multiple sites where it can act to influence milk yield. Hernandez et al. (2008) observed that 5-HT tended to decrease milk lactose percentage but not milk yield.

Selective serotonin reuptake inhibitors (**SSRI**) act to increase extracellular 5-HT concentrations sharply over a short period while acting continually on serotonergic neurotransmissions. The prevention of 5-HT reuptake by SSRI occurs at the presynaptic junction, resulting in increased concentrations of 5-HT in the synaptic cleft (Stahl, 1998). Serotonin has been previously reported to depress lactation in mouse, bovine, and human models (Matsuda et al., 2004; Hernandez et al., 2008; Marshall et al., 2010). Matsuda et al. (2004) reported that 5-HT plays a role in mouse mammary gland development and homeostasis. Hernandez et al. (2008) reported similar findings through an in vitro experiment using cultures of mammary tissue with a lactogenic medium. They observed that 5-HT restricted milk protein mRNA expression in dairy cattle and suggested that 5-HT acts as a negative regulator of lactation.

Specifically, a commonly used SSRI is fluoxetine (**FLX**; Prozac, Eli Lilly & Co., Indianapolis, IN). Local treatment of the lactating mammary gland with FLX resulted in involution of the mammary gland in lactating mice (Marshall et al., 2010). Additionally, a delay was noted in the onset of lactogenesis stage II in women who had taken SSRI during pregnancy and lactation (Marshall et al., 2010).

In lactating animals, measurement of plasma lactose concentration is a common method of determining tight junction (**TJ**) status: if lactose is present, then TJ have

been disrupted (Stelwagen et al., 1999). In humans, treatment of mammary epithelial cells with exogenous 5-HT and FLX caused a biphasic effect on TJ permeability, as low concentrations of 5-HT maintained TJ formation and high concentrations disrupted it (Pai and Horseman, 2008; Marshall et al., 2010). Hernandez et al. (2011) observed that cows treated with intramammary infusions of 5 mg of FLX had greater concentrations of plasma lactose compared with controls, suggesting that FLX caused a disruption of the TJ.

Because of their ability to end lactation, SSRI have been considered for use in the dairy industry to aid in dry-off procedures. Infections in mammary glands are common during the dry-off period and may lead to higher rates of infection in subsequent lactations, resulting in reduced milk production (Eberhart, 1986). Mastitis incidences increase during periods of milk stasis in both dairy animals and humans (Oliver and Smith, 1982; Oliver and Sordillo, 1988; Betzold, 2007). The typical length of milk cessation is 3 d and within these 3 d, as many as 16% of quarters become newly infected (Dingwell et al., 2002). During the dry-off period, the streak canal may not be fully closed, allowing bacteria to enter this portal and create an infection (Cousins et al., 1980). Up to 20% of teat streak canals remain open through wk 6 of the dry period (Williamson et al., 1995; Dingwell et al., 2001). Thus, hastening involution could prevent cases of mastitis during the dry period of dairy animals and maintain subsequent milk production.

We hypothesized that FLX would depress lactation and increase the leakage of lactose into blood serum at dry-off. Our objective was to determine if sheep could be a suitable model to evaluate FLX, administered as an injectable or orally, as a dry-off agent in the dairy industry.

Procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. All experimental procedures were completed at New Mexico State University, Las Cruces (32°19′11″ N, 106°45′55″ W; elevation 1,219 m). Experiment 1 was conducted in April 2010, and experiment 2 was conducted in April 2011. Animals exposed to FLX remained on New Mexico State University's Sheep Unit for at least 5 mo following exposure to FLX. Kim et al. (2004) noted that in pregnant sheep, the elimination half-life of FLX and norfluoxetine (NFLX) is 6.7 and 23 h, respectively.

In experiment 1, 12 Suffolk cross ewes (BW = 83.4 ± 12.2 kg; BCS = 2.1 ± 0.4) in lactation were selected based on similar lambing dates. Ewes received 2.7 kg of chopped alfalfa hay at 0700 h daily and had ad libitum access to water. On approximately d 78 postpartum, lambs were removed from ewes and were kept within sight and hearing distance of their dams; however, they did not share a fence-line.

Experiment 1 used a completely randomized design, as ewes were sorted by number of lambs (single, multiple) and then randomly allotted to treatment. Three treatments were used: 0 mg of FLX (control), 40 mg of FLX, and 80 mg of FLX. Fluoxetine treatments were made by suspending the dose (40 or 80 mg of FLX) in 4 mL of propylene glycol. Control ewes received only a 4-mL injection of propylene glycol. The solution was injected once subcutaneously in the neck of the ewe on d 0. Ewes were penned together in a single dry-lot pen with fence-line bunks following weaning.

Milk production measurements and samples were taken on d 0 (before treatment and immediately after lamb removal) and on d 1, 2, and 3 following weaning at 0800 and 1100 h. Milk production followed the procedure reported by Reynolds and Brown (1991). To initiate milk ejection, a 1-mL intravenous injection of oxytocin (20 USP U/mL; Bimeda-MTC Animal Health Inc., Cambridge, ON, Canada) was given, and hand milking began approximately 1 min after the injection. Once the ewe was milked dry, the ewe remained penned for 3 h. Following the 3-h period, the same procedures to induce milk letdown and milk removal were conducted. The weight of the milk was recorded. Reported milk production values are an estimate of the yield over a 3-h period (**3HMILK**).

In experiment 1, blood samples were collected from the ewe via jugular venipuncture on d 0 (before treatment) and on d 1, 2, and 3 following weaning but before the initiation of lactation, using sterile serum separator tubes (Corvac, Kendall Health Care, St. Louis, MO). Following collection, blood was held at room temperature for 30 to 60 min and then centrifuged (1,500 × g at 4°C for 15 min). Serum was harvested and stored frozen (−20°C) until assayed for lactose. Serum samples from d 2 were evaluated for lactose using an enzymatic assay. The assay measures conversion of lactose into galactose and glucose via the enzyme lactase (Biovision, Mountain View, CA). Assay was performed per manufacturer's instructions using 10 μL of the serum sample.

The MIXED model procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC) was used to analyze 3HMILK and lactose concentrations with ewe as the experimental unit. Lactose concentration was a single measure with diagonal covariant structure and main effect of treatment. Milk production was analyzed as a repeated measure over days where ewe was in the whole plot and the sub-plot was day and day × treatment interaction. The covariant structure for 3HMILK was autoregressive because it was the best fit for our data. If a treatment effect was observed in lactose or