**Technical note: Effects of rumen passage on fluoxetine bioavailability in serum and effects of fluoxetine on serum prolactin concentration and demeanor in ewes**


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**ABSTRACT:** Sheep are becoming increasingly important in medical research. The objective of the present study was to identify changes in bioactivity of fluoxetine during ruminal passage in ewes, and to examine the effects of fluoxetine administration on demeanor and serum prolactin concentration. Twelve mature ewes were administered saline (control), daily oral fluoxetine (40 mg), or alternate-day oral fluoxetine for 10 d. Four additional ewes were fitted with rumen cannulas and administered daily fluoxetine by abomasal deposition. Serum samples were collected daily for 15 d. Serum fluoxetine concentrations (ELISA) were greater \((P < 0.001)\) in ewes in all fluoxetine treatments compared with controls on d 2. Serum fluoxetine concentrations in ewes receiving daily abomasal dosages were greater \((P < 0.007)\) than those in controls on d 2 to 12 and were greater than those in ewes receiving daily or alternate-day oral fluoxetine on d 3 to 12. Serum prolactin concentration (RIA) did not differ \((P = 0.137)\) among treatments and was only weakly correlated with serum fluoxetine concentration \((r = 0.20, P = 0.041)\), and regression analysis revealed that very little variation in serum prolactin concentration was due to serum fluoxetine concentration \((R^2 = 0.04, P = 0.082)\). Demeanor ratings on d 1 to 12 remained at normal in all treatment groups \((P > 0.362)\). However, in ewes that had received an abomasal dosage of fluoxetine, demeanor scores decreased \((P < 0.029)\) on d 13 and 14 before returning to normal on d 15 \((P = 0.397)\). This study indicates that mature ewes may provide a suitable model for the study of fluoxetine, but that a larger oral dosage may be required relative to the human dosage to overcome partial loss of bioactivity during ruminal passage.

**Key words:** ewe, fluoxetine, selective serotonin reuptake inhibitor


Because serotonin is antagonistic to lactation (Hernandez et al., 2008), medical researchers have expressed concern over possible adverse effects of fluoxetine in breast-feeding women (Chambers et al., 1999; Anderson et al., 2003). Because of comparable size and other similarities to humans, sheep have become an increasingly common model for human medical studies, a use that includes fluoxetine research (Morrison et al., 2001; Kim et al., 2004). However, it is unclear if chemical alterations to fluoxetine are made by rumen microbes as the orally administered drug passes through the rumen. Likewise, it is unclear if demeanor is altered in sheep administered fluoxetine. Additionally, increasing serotonin concentrations through administration of fluoxetine may have beneficial implications in sheep and other livestock because cessation of postweaning lactation may be hastened without affecting melatonin or prolactin concentrations (Morrison et al., 2005). Thus,

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the objective of the present study was to establish the detectability of fluoxetine in the serum of sheep given oral or abomasal doses of the drug. Additionally, we sought to determine differences in serum fluoxetine concentrations in ewes administered the drug orally or by deposition directly into the abomasum. Last, we sought to evaluate the effects of the drug on prolactin concentration and observable demeanor. We hypothesized that ruminal passage would diminish the amount of fluoxetine that would be detectible in the blood, but that all sheep receiving the drug would exhibit detectible concentrations.

**MATERIALS AND METHODS**

All procedures involving animals were approved by the New Mexico State University Institutional Animal Care and Use Committee.

**Animals and Experimental Procedure**

Sixteen mature Rambouillet ewes (55 ± 1.9 kg) were examined for health and were shorn. Four of these ewes were fitted with rumen cannulas and allowed a 3-wk recovery period. All ewes were housed in covered 3 × 3 m dry-lot pens (4 ewes per pen), given free access to water, and offered a maintenance ration of alfalfa hay (approximately 2.5 kg/ewe) fed once daily at 0800 h. After a 7-d adjustment period, ewes were administered 1 of 4 treatments (n = 4/treatment): a daily oral drench of fluoxetine (40 mg of fluoxetine hydrochloride in 30 mL of saline, Prozac, Ely Lilly and Co., Indianapolis, IN), a daily abomasal dosage of fluoxetine (40 mg of fluoxetine hydrochloride in 30 mL of saline), an alternate-day oral drench of fluoxetine (40 mg of fluoxetine hydrochloride in 30 mL of saline), or a daily drench of saline carrier (30 mL of physiological saline). Briefly, abomasal dosage occurred as a 1-min bolus through Tygon infusion lines (i.d. 0.5 cm, o.d. 0.6 cm, wall 0.1 cm, formula R-3603, Cole Palmer Instr. Co., Vernon Hills, IL). One end of the line was inserted manually through the reticulo-omasal sphincter, which was made accessible through the rumen cannula, as described by van Nolte et al. (2008), with the opposite end of the line exteriorized through the rumen cannula. Lines were held in the abomasum by 5-cm rubber flanges. Treatments were applied at 0700 h beginning on d 1 and were discontinued after d 10. During this 10-d period, daily blood samples were collected immediately before treatment administration. Additionally, daily blood samples were collected for 5 d after treatment ceased.

**Sample Collection and Analysis**

Blood samples were collected via jugular venipuncture into 10-mL vacuum tubes (Corvac serum-separator, Kendall Health Care, St. Louis, MO). Blood samples were kept at room temperature for 30 to 60 min and were centrifuged (1,500 × g at 4°C for 15 min). After centrifugation, serum was stored in plastic vials at −80°C until assayed. Serum fluoxetine was quantified using a commercially available ELISA kit (Neogen Corp., Lexington, KY; Heller et al., 1998). To verify its performance, a pool of serum was obtained as described previously from ewes orally administered fluoxetine (40 mg) daily. Addition of 5, 10, 15, 20, 25, and 30 µL of this serum resulted in displacement parallel to the standard curve (Figure 1). Ovine serum was also compared with pools of ovine saliva and ovine rumen fluid as additional validation. An additional serum pool collected from ewes not administered fluoxetine was used to prepare spiked samples containing 10 and 20 ng of fluoxetine (Neogen Corp.) per milliliter of serum. When 20-µL aliquots of these samples were assayed in duplicate, 9.7 (97% recovery) and 18.5 (93% recovery) ng/mL were obtained, respectively. Sensitivity of the assay (95% of maximum binding) was 5.5 ng/mL. The assay had an intraassay CV of 11.7%. Serum prolactin was quantified in samples collected on d 3 to 5 and d 9 to 11 by double-antibody RIA, as described by Spoon and Hallford (1989). Intra- and interassay CV for serum prolactin determinations were less than 7%. The demeanor of each individual ewe was rated on a 4-point Likert scale derived from behavioral indicators of depression or other abnormal behavior just before and during feeding. Each ewe was given a score of 4 if no deviation from typical behavior was noted, whereas scores increasingly less than 4 were given to ewes with behavior that was increasingly deviated from normal behavior. Behavioral observations were based on social and physical cues, including movement and activity, attentiveness, head posturing, and assertiveness during feeding. Initial rating scores were established and agreed on by 2 independent raters. In addition to demeanor ratings, experimenters monitored the occurrence of common side effects identified in human subjects taking fluoxetine (Stahl et al., 1997). However, none of the monitored side effects was observed.

**Statistical Analyses**

The experimental design was completely randomized and ewe was the experimental unit. Serum fluoxetine and prolactin concentrations were analyzed using the MIXED (mixed-models) procedure (SAS Inst. Inc., Cary, NC) with a repeated-measures function. Serum prolactin concentrations were considered to be early stage (d 3 to 5) or later stage (d 9 to 11), and these periods were analyzed separately. Where a treatment × day interaction was detected, treatment effects were examined within day. The relationship between serum fluoxetine and prolactin concentration was examined using the REG (regression) procedure of SAS. The observed demeanor score was analyzed using the FREQ (frequency) procedure of SAS with a χ² function.
RESULTS AND DISCUSSION

An interaction was observed ($P = 0.006$) between treatment and day for serum fluoxetine concentration; thus, treatment effects were examined within each day. Because blood samples were taken before treatment administration each day, serum fluoxetine concentration (Figure 2) did not differ ($P = 0.997$) among treatments on d 1, as expected. However, serum fluoxetine was greater ($P < 0.001$) in all fluoxetine treatments compared with controls on d 2. Serum fluoxetine concentrations in ewes receiving daily abomasal dosages of the compound remained greater ($P < 0.007$) than those of controls from d 2 to 12 and were also greater than those of ewes receiving daily or alternate-day oral fluoxetine treatment on d 3 to 12. This observation indicates greater bioavailability of fluoxetine in circulation when the rumen is bypassed during administration. Although Redshaw et al. (2008) found that most environmental microbes do not easily digest fluoxetine, Chaubey et al. (2006) reported that bacteria of the genus *Arthrobacter* are capable of breaking down the drug. Interestingly, *Arthrobacter* are generally among the mixture of microflora that populates the rumen (Bagnyuk et al., 1985). Additional work is needed to determine if other ruminal bacteria possess similar digestive abilities, although *Bacillus*, *Pseudomonas*, and *Streptomyces* may be candidate genera. Surprisingly, ewes receiving daily or alternate-day oral fluoxetine differed ($P < 0.050$) in serum fluoxetine concentration only on d 10, and were similar on all other days. Similarities in serum fluoxetine between ewes in these 2 groups may be due to efficient postingestive storage of the compound, coupled with a lengthy half-life, because enzymatic metabolism of fluoxetine in the liver is slow (Burke et al., 2000). However, both daily and alternate-day orally dosed ewes exhibited increased ($P < 0.002$) serum fluoxetine concentrations compared with controls on d 3, 6, and 7, and ewes administered oral treatment daily also exhibited increased serum fluoxetine compared with controls on d 5 and 10. This finding indicates that an observable amount of fluoxetine was escaping the rumen environment in an intact, bioactive form. However, when using sheep as a model for human medical research, the ruminant animal may require a larger oral dose of fluoxetine than a nonruminant to achieve similar serum concentrations, as indicated by greater serum concentrations of fluoxetine when the rumen was bypassed by direct abomasal dosage. No treatment effect ($P > 0.05$) was observed after d 13, indicating that all stores of fluoxetine had been depleted and cleared from the body of the animals by this time.

No treatment $\times$ day interaction was observed ($P > 0.51$) for serum prolactin concentrations in either early-stage (d 3 to 5 of treatment) or later-stage (d 9 to 11 of treatment) samples. In early-stage samples, serum prolactin concentrations did not differ ($P > 0.137$) among fluoxetine treatments or among days (Table 1). This finding supports previous research that indicates fluoxetine has no observable effect on prolactin (Morrison...
et al., 2005) and suggests that effects of the former on lactation may be independent of the latter. However, serum prolactin concentrations in later-stage samples were less \( (P < 0.029) \) in ewes receiving alternate-day oral doses compared with those receiving any other fluoxetine treatment. Reduced prolactin concentrations in ewes receiving fluoxetine orally every other day compared with ewes receiving daily oral or abomasally infused fluoxetine would appear to support earlier findings of increased prolactin concentrations in humans (Urban and Veldhuis, 1991) and monkeys (Pecins-Thompson and Bethea, 1997) after fluoxetine administration because these ewes were administered less fluoxetine over the sampling period. However, serum prolactin concentrations did not differ \( (P > 0.05) \) between ewes receiving daily oral or abomasally infused fluoxetine compared with control ewes. Additionally, serum prolactin concentration was only weakly correlated with serum fluoxetine concentration \( (r = 0.20, P = 0.041) \), and regression analysis revealed that very little variation in serum prolactin concentration was due to serum fluoxetine concentration \( (R^2 = 0.04, P = 0.082) \). These observations, coupled with a lack of difference between serum fluoxetine concentrations in ewes receiving either daily or alternate-day oral dosages of the drug, seem to indicate that fluoxetine had little or no effect on circulating prolactin concentrations in ewes, as reported by Morrison et al. (2005). As with early-stage samples, serum prolactin concentrations in later-stage samples did not differ \( (P = 0.51) \) among days.

Demeanor ratings throughout fluoxetine administration (d 1 through 10) and the first 2 d after withdrawal (d 11 and 12) remained at the normal rating of 4 across all treatment groups \( (P > 0.36) \). However, in ewes receiving an abomasal dosage of fluoxetine, demeanor scores decreased \( (P < 0.029) \) on d 13 and 14 before returning to normal on d 15 \( (P = 0.397) \). 5 d after cessation of the fluoxetine treatment. Although severe side effects, such as depression, suicidal attempts, and suicidal deaths, have been shown to be relatively rare in human subjects taking second-generation antidepressant medications such as fluoxetine (Simon et al., 2006; Gartlehner et al., 2008), other research indicates that fluoxetine may be associated with noticeable changes in demeanor (Breggin, 2004). In addition to concurrent side effects, depression and flu-like symptoms have occurred on cessation of fluoxetine use (Stahl et al., 1997). In the present study, no observable side effects were identified during the administration of fluoxetine. However, on withdrawal of the medication, ewes that had received an abomasal dosage of fluoxetine exhibited a noticeable decline from normal behavior, as indicated by decreased activity, lowered head posturing, and decreased appetite and assertiveness during feed-

Figure 2. Serum fluoxetine concentration in ewes receiving fluoxetine (40 mg, Prozac, Ely Lilly and Co., Indianapolis, IN) daily by oral drench or abomasal deposition or receiving fluoxetine on alternate days by oral drench from d 1 to 10. A treatment × day interaction was observed \( (P = 0.006) \). Differing treatment means within each day are denoted by differing letters \( (a-c; P < 0.05) \).
Because fluoxetine has a relatively long half-life in circulation and has been shown to remain in the body for days or weeks after the drug is no longer taken, and because the clearance rate from the body is generally inversely proportional to the length of time spent taking the drug (Stahl et al., 1997), serum fluoxetine should have decreased at the same rate in all treatment groups. However, abomasally infused ewes exhibited a more sudden decline in serum fluoxetine concentration compared with the other treatment groups, especially between d 12 and 13. This rapid decrease may have contributed to the observed behavioral changes on d 13 and 14.

Data from the present study indicate that passage through the rumen of sheep decreases the bioavailability of fluoxetine. However, an observable amount of the drug does reach circulation in the active form. Fluoxetine administration appeared to have little or no effect on serum prolactin concentration, and demeanor did not change in ewes during administration of the drug. Demeanor did decline noticeably in ewes that had received abomasal dosage of the drug. This change followed discontinuation of treatment by 3 d and appeared to coincide with a rapid decline in serum fluoxetine concentrations. However, by 5 d after discontinuation, the demeanor of these ewes was no longer different from that of other treatment groups. This study indicates that mature ewes may provide a suitable model for the study of fluoxetine, but that oral dosage may need to be increased relative to human dosage to overcome partial loss of bioactivity during ruminal passage.

Table 1. Serum prolactin concentrations (ng/mL) for early- and late-stage periods in ewes receiving fluoxetine\(^1\) (40 mg) daily by oral drench or abomasal deposition or on alternate days by oral drench from d 1 to 10

<table>
<thead>
<tr>
<th>Period</th>
<th>Control</th>
<th>Abomasally infused</th>
<th>Daily oral</th>
<th>Alternate-day oral</th>
<th>SE(^2)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early stage(^3)</td>
<td>15.5</td>
<td>27.7</td>
<td>25.4</td>
<td>10.1</td>
<td>6.1</td>
<td>0.137</td>
</tr>
<tr>
<td>Late stage(^4)</td>
<td>31.2(^a)</td>
<td>34.3(^a)</td>
<td>31.9(^a)</td>
<td>15.3(^b)</td>
<td>6.8</td>
<td>0.029</td>
</tr>
</tbody>
</table>

\(^a\)Means with differing superscripts differ (P < 0.05).
\(^b\)Prozac (Ely Lilly and Co., Indianapolis, IN).
\(^2\)n = 4.
\(^3\)Means of d 3, 4, and 5 (treatment x day, P = 0.852; day effect, P = 0.822).
\(^4\)Means of d 9, 10, and 11 (treatment x day, P = 0.51; day effect, P = 0.50).
LITERATURE CITED


