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Comparison of Effects of Capromorelin and Mirtazapine on Appetite in New Zealand White Rabbits (*Oryctolagus cuniculus*)

Janna MH Draper,[1,*] Daniel J Savson,[1] Elizabeth S Lavin,[1] Erica R Feldman,[1] Bhupinder Singh,[1] Manuel Martin-Flores,[2] and Erin K Daugherity[1]

Inappetence is a welfare concern in rabbits (*Oryctolagus cuniculus*), as it can lead to potentially fatal gastrointestinal stasis. In other species, inappetence is commonly treated with appetite stimulants; however, few published studies have evaluated the efficacy of appetite stimulants in rabbits. We performed 2 studies to evaluate the effects of capromorelin and mirtazapine on appetite in New Zealand White (NZW) rabbits. In the first study, healthy rabbits (*n* = 9) were evaluated using a randomized crossover design and 9 treatments: capromorelin 4 mg/kg oral (PO) once a day (SID), capromorelin 8 mg/kg PO SID, saline control PO SID, capromorelin 4 mg/kg PO twice a day (BID), capromorelin 8 mg/kg PO BID, saline control PO BID, mirtazapine 0.5 mg/kg transdermal (TD) SID, mirtazapine 1 mg/kg TD SID, and saline control TD SID for 3 d with a 1-wk washout period between treatments. Treatment efficacy was assessed by measuring daily feed intake and fecal output and by weighing rabbits twice a week. Overall, feed intake and fecal output were higher for all treatments as compared with controls, except for fecal output in the capromorelin 4 mg/kg and 8 mg/kg PO SID groups. Feed intake and fecal output were significantly higher with mirtazapine as compared with capromorelin. Body weight and erythema/petechia of the pinnae were greater in the mirtazapine 1 mg/kg TD SID group than in the control group. A second study evaluated rabbits that had undergone surgery (castration, *n* = 7) and then received one of 3 treatments: capromorelin 8 mg/kg PO BID, mirtazapine 1 mg/kg TD SID, or saline PO BID for 3 d postoperatively. Feed intake and fecal output in the postoperative mirtazapine group were not significantly different from those of the capromorelin and control groups. Due to its superior efficacy as compared with capromorelin in healthy NZW rabbits, we recommend considering mirtazapine as a treatment for inappetence in NZW rabbits.

Abbreviations and Acronyms: BID, twice daily; GI, gastrointestinal; NZW, New Zealand White; SID, once daily; TD, transdermal

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Introduction

Rabbits are a commonly used research species, with approximately 142,000 rabbits used for research purposes in the United States in 2019 according to the USDA's annual animal usage report.[3] They are used to study immunology, cancer, reproduction, arthritis, and cardiovascular health.[8,10] Inappetence can occur in rabbits as a result of stress, pain, and/or drug administration.[9,13,20] For example, buprenorphine, a partial mu agonist, is a commonly used analgesic in rabbits that decreases feed intake and fecal output and prolongs gastrointestinal (GI) transit time.[9,13,17] Inappetence is especially dangerous in rabbits because it can lead to GI stasis.[19] Rabbits are prone to GI stasis due to high energy demands and reliance on indigestible fiber for peristalsis.[19] Proliferation of harmful GI bacteria and intestinal blockages may occur during GI stasis, which can result in death.[19] Current standard-of-care treatments for inappetence and GI stasis include fluid therapy, enteral nutrition, and gastrointestinal motility stimulants.[9,19] However, cisapride, a commonly used GI motility stimulant used in multiple domestic species, was not effective for treating opioid-induced GI stasis in rabbits.[9] Therefore, additional studies assessing refined or novel treatments of inappetence and GI stasis in rabbits are warranted.

Appetite stimulants, such as capromorelin and mirtazapine, are commonly used to treat inappetence in dogs and cats.[4,5,14,18,21-26] Capromorelin is a growth hormone secretagogue receptor (GHS-R) agonist that mimics the hunger hormone ghrelin.[21,23-26] Ghrelin is secreted from the cells of the stomach and binds to growth hormone secretagogue receptor 1a (GHS-R1a) in the hypothalamus and pituitary gland, causing the feeling of hunger.[21,23-26] Ghrelin is highly conserved across mammalian species.[21] Formulations of capromorelin such as Entyce and Elura are FDA-approved for stimulation of appetite and management of weight loss in dogs and cats respectively.[21-26] Another appetite stimulant is mirtazapine, an α2-adrenergic receptor antagonist, nor-adrenergic and serotonergic antidepressant drug.[14] The mechanism of appetite stimulation is unknown, but is thought to be multifactorial, involving the antagonism of serotonin receptors (5-HT2 and 5-HT3), inhibition of histamine (H1) receptors, and changes in leptin and tumor necrosis factor α (TNF-α).[14,18] Histamine is highly conserved across most vertebrate species.[16] Mirtazapine is available as an oral and transdermal (TD) formulation.[4,5,15,19] A recent study found that oral mirtazapine increased fecal output in NZW rabbits but had no effect on feed intake.[15] Due to the difficulty administering oral medication to cats, transdermal formulations increase owner compliance with drug administration as it is simpler to

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[1]Center for Animal Resources and Education, and [2]Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York
[*]Corresponding author. Email: draperjanna9@gmail.com

use than the oral formulation in the home environment.[4,5,14,18] Given the effectiveness of capromorelin and mirtazapine in dogs and cats, we assessed their effectiveness at increasing appetite in rabbits.

The current investigation compared the effects of oral capromorelin and transdermal mirtazapine on appetite in healthy (study one) and postoperative (study 2, post-castration) NZW rabbits to assess their potential use in the treatment of inappetence in rabbits. Treatment efficacy was assessed by measuring feed intake, fecal output, and body weight. We hypothesized that capromorelin and mirtazapine would increase appetite in both healthy and postoperative NZW rabbits, evidenced by increased feed intake, fecal output, and body weight.

Materials and Methods

Animals. Heathy, adult, intact male NZW rabbits (*Oryctolagus cuniculus*; $n = 9$; age: 3 mo; weight; 2.9 to 3.2 kgs) were purchased from Charles River Laboratories (Wilmington, MA). Rabbits were negative for *Pasteurella* spp., *Helicobacter* spp., *Bordetella bronchiseptica, Salmonella* spp., *Clostridium piliforme, Lawsonia* spp., *Treponema* spp., cilia-associated respiratory bacillus, *Eimeria* spp., *Passalurus ambiguus*, *Cheyletiella parasitovorax*, *Psoroptes cuniculi*, *Leporacarus gibbus*, reovirus, rabbit hemorrhagic disease virus, lymphocytic choriomeningitis virus, and rotavirus. Animals were housed in an AAALAC-accredited facility in compliance with the Animal Welfare Act and The *Guide for the Care and Use of Laboratory Animals*.[1,12] All work was approved by the Institutional Animal Care and Use Committee (IACUC) of Cornell University (Ithaca, NY).

Rabbits were acclimated for 2 wk prior to study initiation. Rabbits were housed individually in stainless-steel cages (Allentown, NJ) with $^{3}/_{8}$-in (9.5 mm) stainless-steel linear slotted floors to allow collection of feces from a tray under the cage. Fresh paper liners were placed in the tray under the cages daily when on study to aid in urine absorption and facilitate easy fecal collection. The cages were cleaned biweekly in a rack washer that reached 180 °F (82.2 °C). Standard husbandry practices were not expected to impact fecal weights or other physiologic parameters. Rabbits were moved out of the cages at least once per week for cleaning or movement to floor pens. Rabbits were given stainless-steel mailboxes (Wheaton Fabrication, Ithaca, NY) for shelter, and stainless-steel or plastic toys as enrichment. Rabbits were fed a nutritionally complete pelleted diet (Country Feeds 16% Rabbit Feed, Nutrena, Minneapolis, MN) in bowls, and municipal tap water was provided without restriction in glass water bottles or water bowls depending on rabbit preference. No significant difference in water intake was expected thehe method of water delivery. All cage components were cleaned weekly in a tunnel washer that reached 180 °F (82.2 °C). The housing room was maintained at 66 to 70 °F (18.9 to 21.1 °C), relative humidity of 30% to 70% and on a 12:12-h light:dark cycle with fluorescent lights at 30 foot candles (FC) or 322.9 lux. When not on study and during washout periods, rabbits received timothy hay (Western timothy hay; crude fiber maximum, 32%, Oxbow, Murdock, NE) and were housed on the floor in ground pens. Timothy hay was not offered when rabbits were on study due to our inability to accurately measure consumption.

Experimental design. ***Study One: Comparison of the effects of capromorelin and mirtazapine on appetite in healthy NZW rabbits.*** Rabbits ($n = 9$) received 9 treatments in a crossover, randomized design, with at least a one-week washout period between treatments. The reported half-life of oral capromorelin in dogs is 1.2 h while the reported half-life of transdermal mirtazapine in cats is variable but is reported to be up to is 26.8 h. Thus, a one-week washout period was chosen as suitable for complete elimination of the drugs.[5,14,21,25] Because oral capromorelin has vanilla flavoring, compounding the drug was deemed unnecessary. Each rabbit was randomly assigned a treatment prior to each study period over an 18-wk period. No rabbit received the same treatment more than once. The treatment groups included: SID oral medications—capromorelin 4 mg/kg PO SID (Aratana Therapeutics, Leawood, KS), capromorelin 8 mg/kg PO SID, saline control PO SID (Hospira, Lake Forest, IL); BID oral medications—capromorelin 4 mg/kg PO BID, capromorelin 8 mg/kg PO BID, saline control PO BID; and SID transdermal medications—mirtazapine 0.5 mg/kg TD SID (Dechra Veterinary Products, Overland Park, KS), mirtazapine 1 mg/kg TD SID, and saline control TD SID. Saline was dosed at the same volume as the treatments. All treatments were administered for 72 h. SID treatments were administered in the morning, and BID treatments administered in the morning and again at least 8 h after morning treatment. For oral treatments, one to 2 skilled handlers removed and restrained rabbits and administered treatments. Oral capromorelin was administered via a syringe into the corner of the mouth. Rabbits remained in the home cage when receiving transdermal treatments to reflect real life practices. Transdermal mirtazapine was applied using a 1-mL syringe to ensure accurate dosing. Consistent with manufacture recommendations, disposable gloves were worn during administration to the ear to protect personnel from transdermal absorption. Mirtazapine was applied in alternate ears each day. The ear was not cleaned or shaved before application. The dosages for capromorelin and mirtazapine were extrapolated from formularies that included clinical experience and pilot data.[2,4,5,11,14,17,18,21,23-26]

Data were collected for 5 d during each treatment period. On day one (the first day of treatment), each rabbit was weighed and received a baseline feed amount (400 g) in bowls, and fecal trays were emptied. Rabbits received treatment on days one through 3. Feed and feces were weighed daily on days 2 through 5 at approximately the same time every day. If cecotrophs were present in the tray, they were included in the total fecal weight. After the daily weighing, the feed was replenished to 400 g and fecal trays cleaned. Rabbits were weighed again on day 4. The rabbits were monitored daily by a veterinarian and husbandry staff for adverse clinical effects, such as signs of reduced feed or water intake or fecal output, change in behavior, and lower body condition. Starting during the second treatment week, the ears of rabbits receiving transdermal treatments were photographed daily for ear scoring (Figure 1).

Study Two: Comparison of the effects of capromorelin and mirtazapine on postoperative NZW rabbits. The same rabbits used in study one received one of 3 treatments after castration surgery in a randomized design: capromorelin 8 mg/kg PO BID ($n = 2$), mirtazapine 1 mg/kg TD SID ($n = 3$), and saline control PO BID ($n = 2$). The saline control was given at the same volume as for capromorelin treatment. On day one (the first day of treatment), each rabbit underwent surgical castration and received buprenorphine (0.03 mg/kg SQ every 6 to 8 h; buprenorphine hydrochloride injection, Par Pharmaceutical, Chestnut Ridge, NY) and meloxicam (1 mg/kg SQ; Boehringer Ingelheim Animal Health USA INC., Duluth, GA; or PO every 24 h; Aspen Veterinary Resources, LTD, Liberty, MO) for 3 d.[6] Rabbits were castrated prior to adoption; adoption-related castrations are covered under clinical protocols, unrelated to this protocol. Two rabbits that developed complications after surgery, unrelated to study one,

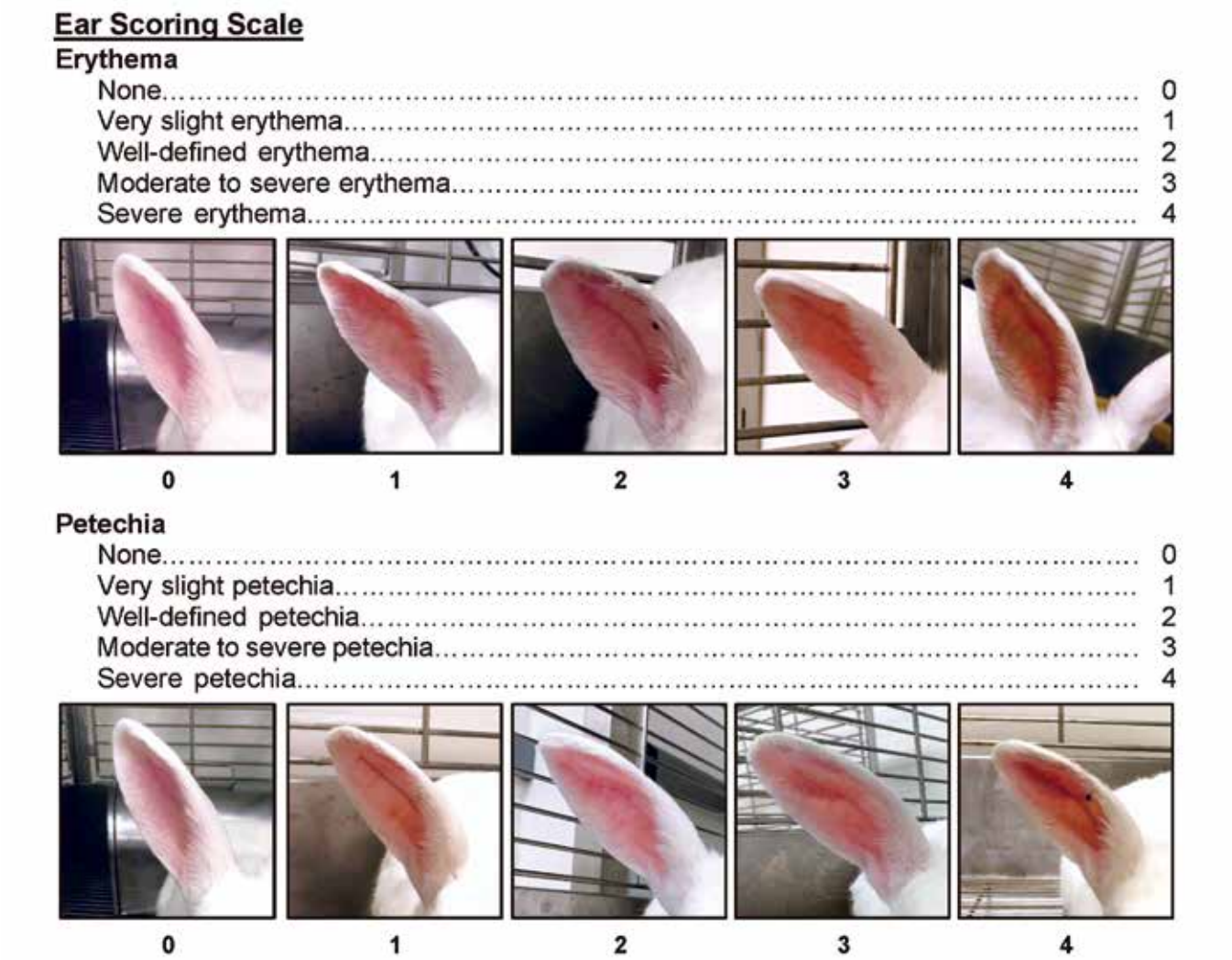

Figure 1. Ear scoring scale used to score erythema and petechia of ears from rabbits receiving transdermal treatments in study 1. Erythema and petechia scores were combined for a total ear score.

were excluded from study 2. The dosing schedule for study 2 was the same as that used in study one.

A scrotal approach was used for castration. Rabbits were ansethetized using injections of butorphanol (0.5 mg/kg IM), dexmedetomidine (60 mcg/kg IM), ketamine (0.5 mg/kg IM), and inhalational isoflurane if needed. The incisions were closed with skin glue to reduce secondary irritation due to suture material and to eliminate the need for another sedation event for suture removal.[19] Antisedan (equal volume to the dexmedetomidine) was used for reversal. After recovery from anesthesia, defined as normal ambulation, the rabbits were weighed, received a baseline amount of food (400 g) in bowls, and fecal trays were emptied. Rabbits received the first treatment of capromorelin, mirtazapine, or saline after recovery from anesthesia, so the exact time of day that each rabbit received the treatment varied within the 72-h treatment period. The treatment period was the same in both studies. Data were collected in study 2 in the same manner as in study 1.

Measurement of body weight, feed intake, fecal output, daily observations, and ear scoring. In both studies, body weights were obtained on days 1 and 4. Feed and feces were collected and weighed daily in the morning on days 2 through 5. Feed found in the fecal trays was also collected and weighed. Daily observations and monitoring for possible adverse drug reactions or clinical signs occurred daily on days 1 through 5. Beginning during the second treatment week of study one, photographs of both ears of rabbits receiving transdermal treatments were collected to assess cutaneous responses to the transdermal treatments. Photographs were taken immediately before treatment, at 10 to 30 min after treatment, and at 7 to 8 h after treatment on days one through 3. On days 4 and 5, one photograph was taken in the morning. Three veterinarians who were blind to treatments used an ear scoring scale to score ear photographs for erythema and petechia on a scale of 0 to 4 (Figure 1). Erythema and petechia scores were combined for a total ear score, and daily averages were calculated for each treatment group.

Statistical analysis. For study one, change in body weight (day one to day 4) was analyzed using a linear mixed effect model with a fixed effect of treatment and a random effect of rabbit due to the repeated measurements taken on rabbits across treatments. Feed intake, fecal output, and daily total ear scores were analyzed using linear mixed effects models with fixed effects

of the treatment, day, and an interaction between the treatment and day. Random effects of rabbit ID and rabbit ID nested in the treatment were used to control for repeated measurements across the treatments and across days within each treatment. Significance of the fixed effects was tested using F tests with a Satterthwaite approximation for the degrees of freedom and pairwise comparisons were made using Tukey's HSD method to control the Type 1 error rate. Model assumptions were assessed by visual assessment of the residuals. The data is expressed as boxplots representing the median and interquartile range (IQR) with any individual points outside the IQR marked as dots. Statistical significance was indicated with a p value equal to or less than 0.05.

For study 2, change in body weight (day one to day 4) was analyzed using a one-way ANOVA with a main effect of treatment. Feed intake and fecal output were analyzed using linear mixed effects models with fixed effects of the treatment, day, and an interaction between the treatment and day, and a random effect of rabbit ID due to the repeated measurements taken on rabbits across days. Significance of the fixed effects was tested using F tests with a Satterthwaite approximation for the degrees of freedom, and pairwise comparisons were made using Tukey HSD method to control the Type 1 error rate. Model assumptions were assessed by visual assessment of the residuals.

Results

Study One: Effects of capromorelin and mirtazapine on appetite in healthy NZW rabbits. ***Effects of capromorelin and mirtazapine on feed consumption.*** Administration of capromorelin 4 mg/kg PO SID was associated with significant increases in feed consumption on days 2 ($P = 0.0167$), 3 ($P = 0.0326$), and 4 ($P = 0.0136$), as was administration of capromorelin 8 mg/kg PO SID on day 3 ($P = 0.0129$) as compared with the saline control PO SID (Figure 2). For the BID oral treatments, a significant increase in feed consumption was detected for rabbits given capromorelin 4 mg/kg PO BID on day 4 ($P = 0.0006$) and capromorelin 8 mg/kg PO BID on days 3 ($P = 0.0246$) and 4

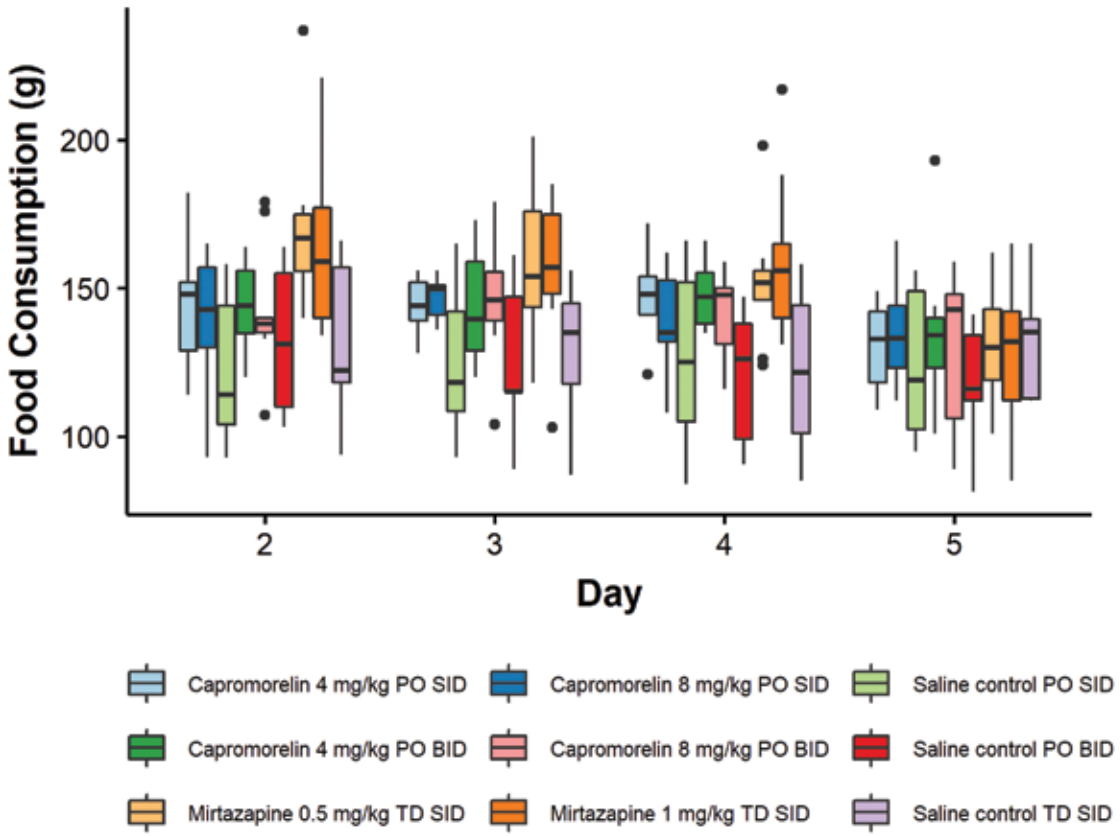

Figure 2. Feed intake in grams, as defined by amount consumed over 24 h, in 9 male, NZW rabbits that received treatments on days 1 to 3. Boxplots represent the median and interquartile range (IQR), and any individual points outside the IQR are marked as dots. On day 2, feed intake was significantly greater for capromorelin 4 mg/kg PO SID as compared with the saline control PO SID ($P = 0.0167$); mirtazapine 0.5 mg/kg transdermal TD SID as compared with saline control TD SID ($P < 0.0001$); mirtazapine 1.0 mg/kg TD SID as compared with saline control TD SID ($P < 0.0001$); mirtazapine 0.5 mg/kg TD SID compared with capromorelin 4 mg/kg PO SID ($P = 0.0009$) and BID ($P = 0.0011$); and mirtazapine 1 mg/kg TD SID compared with capromorelin 8 mg/kg PO SID ($P = 0.0006$) and BID ($P = 0.0019$). On day 3, there was a significant increase in feed intake in capromorelin 4 mg/kg PO SID when compared with the saline control PO SID ($P = 0.0326$); capromorelin 8 mg/kg PO SID compared with the saline control PO SID ($P = 0.0129$); capromorelin 8 mg/kg PO BID compared with saline control PO BID ($P = 0.0246$); mirtazapine 0.5 mg/kg TD SID compared with saline control TD SID ($P = 0.0007$); and mirtazapine 1 mg/kg TD SID compared with saline control TD SID ($P = 0.0011$). On day 4, there was a significant increase in feed intake in capromorelin 4 mg/kg PO SID when compared with the saline control PO SID ($P = 0.0136$); capromorelin 4 mg/kg PO BID compared with saline control PO BID ($P = 0.0006$); capromorelin 8 mg/kg PO BID compared with saline control PO BID ($P = 0.019$); mirtazapine 0.5 mg/kg TD SID compared with saline control TD SID ($P = 0.0004$); mirtazapine 1 mg/kg TD SID compared with saline control TD SID ($P < 0.0001$); and mirtazapine 1 mg/kg TD SID compared with capromorelin 8 mg/kg PO SID ($P = 0.0115$) and BID ($P = 0.0251$). On day 5, there was no significance differences between treatments.

(P = 0.0190) as compared with the saline control PO BID (Figure 2). Rabbits given mirtazapine 0.5 mg/kg TD SID showed a significant increase in feed intake on days 2 (P < 0.0001), 3 (P = 0.0007) and 4 (P = 0.0004), and those given mirtazapine 1 mg/kg TD SID showed significant effects on days 2 (P < 0.0001), 3 (P = 0.0011) and 4 (P < 0.0001) as compared with saline control TD SID (Figure 2). When comparing all the treatments, feed intake increased significantly for rabbits given mirtazapine 0.5 mg/kg TD SID as compared with capromorelin 4 mg/kg PO SID (P = 0.0009) and BID (P = 0.0011) on day 2. Feed intake was significantly higher in rabbits given mirtazapine 1 mg/kg TD SID as compared with capromorelin 8 mg/kg PO SID (P = 0.0006) and BID (P = 0.0019) on day 2. Feed intake was significantly higher in rabbits given mirtazapine 1 mg/kg TD SID as compared with capromorelin 8 mg/kg PO SID (P = 0.0115) and BID (P = 0.0251) on day 4 (Figure 2).

Effects of capromorelin and mirtazapine on fecal output. Rabbits given oral SID treatments showed no significant increases in fecal output between treatments at any time point (Figure 3). Oral BID treatments were associated with a significantly greater fecal output in rabbits given capromorelin 4 mg/kg PO BID (P = 0.0229) and 8 mg/kg PO BID (P = 0.0359) on day 4 as compared with the saline control PO BID (Figure 3). Rabbits given transdermal treatments of mirtazapine 0.5 mg/kg TD SID showed a significantly greater fecal output on days 2 (P = 0.0171), 3 (P < 0.0001), and 4 (P = 0.0015), and rabbits given mirtazapine 1.0 mg/kg TD SID were significantly greats on days 3 (P = 0.0001) and 4 (P < 0.0001) as compared with the saline control TD SID (Figure 3). A comparison of all treatments showed a significantly greater in fecal output in rabbits given mirtazapine 0.5 mg/kg TD SID as compared with capromorelin 4 mg/kg PO SID on days 2 (P = 0.019) and 3 (P = 0.0218). Mirtazapine 0.5 mg/kg TD SID produced a significantly greater fecal output as compared with capromorelin 4 mg/kg PO BID on day 3 (P = 0.0203). The mirtazapine 1 mg/kg TD SID group showed a significantly greater fecal output as compared with the capromorelin 8 mg/kg PO SID group on day 4 (P = 0.014) (Figure 3).

Effects of capromorelin and mirtazapine on body weight. A comparison that included all treatments showed a significantly greater body weight in rabbits given mirtazapine 1 mg/kg TD SID (P = 0.0019) as compared with saline control TD SID. No other significant differences in weight were seen for the other treatment comparisons (Figure 4).

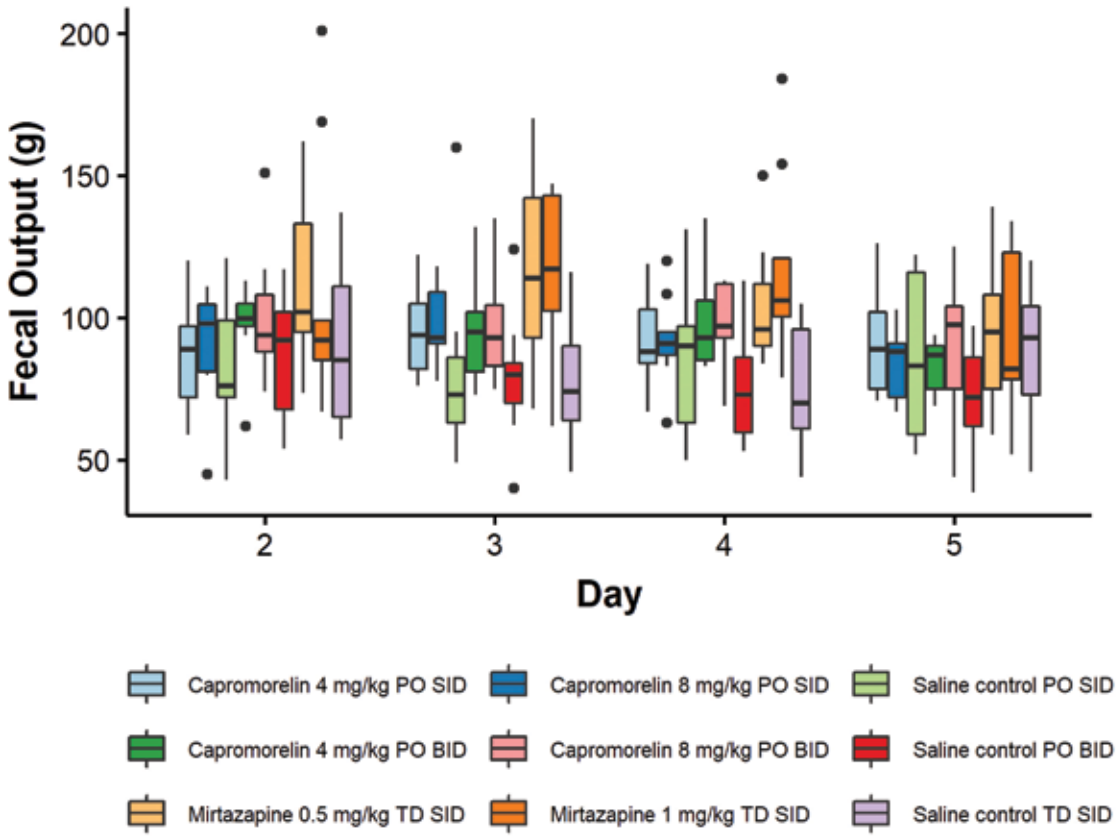

Figure 3. Fecal output in grams, as defined by amount defecated over 24 h, in 9 male, NZW rabbits that received treatments (n = 9) on days 1 to 3. Boxplots represent the median and interquartile range (IQR) with any individual points outside the IQR marked as dots. On day 2, there was a significant increase in fecal output in mirtazapine 0.5 mg/kg transdermal (TD) SID compared with saline control TD SID (P = 0.0171); and mirtazapine 0.5 mg/kg TD SID compared with capromorelin 4 mg/kg PO SID (P = 0.019). On day 3, there was a significant increase in fecal output mirtazapine 0.5 mg/kg TD SID compared with saline control TD SID (P < 0.0001); mirtazapine 1.0 mg/kg TD SID compared with saline control TD SID (P = 0.0001); mirtazapine 0.5 mg/kg TD SID compared with capromorelin 4 mg/kg PO SID (P = 0.0218); and mirtazapine 0.5 mg/kg TD SID compared with capromorelin 4 mg/kg PO BID (P = 0.0203). On day 4, there was a significant increase in fecal output in capromorelin 4 mg/kg PO BID compared with control PO BID (P = 0.0229); capromorelin 8 mg/kg PO BID compared with control PO BID (P = 0.0359); mirtazapine 0.5 mg/kg TD SID compared with saline control TD SID (P = 0.0015); mirtazapine 1.0 mg/kg TD SID compared with saline control TD SID (P < 0.0001); and mirtazapine 1 mg/kg TD SID compared with capromorelin 8 mg/kg PO SID (P = 0.014). On day 5, there was no significance differences between treatments.

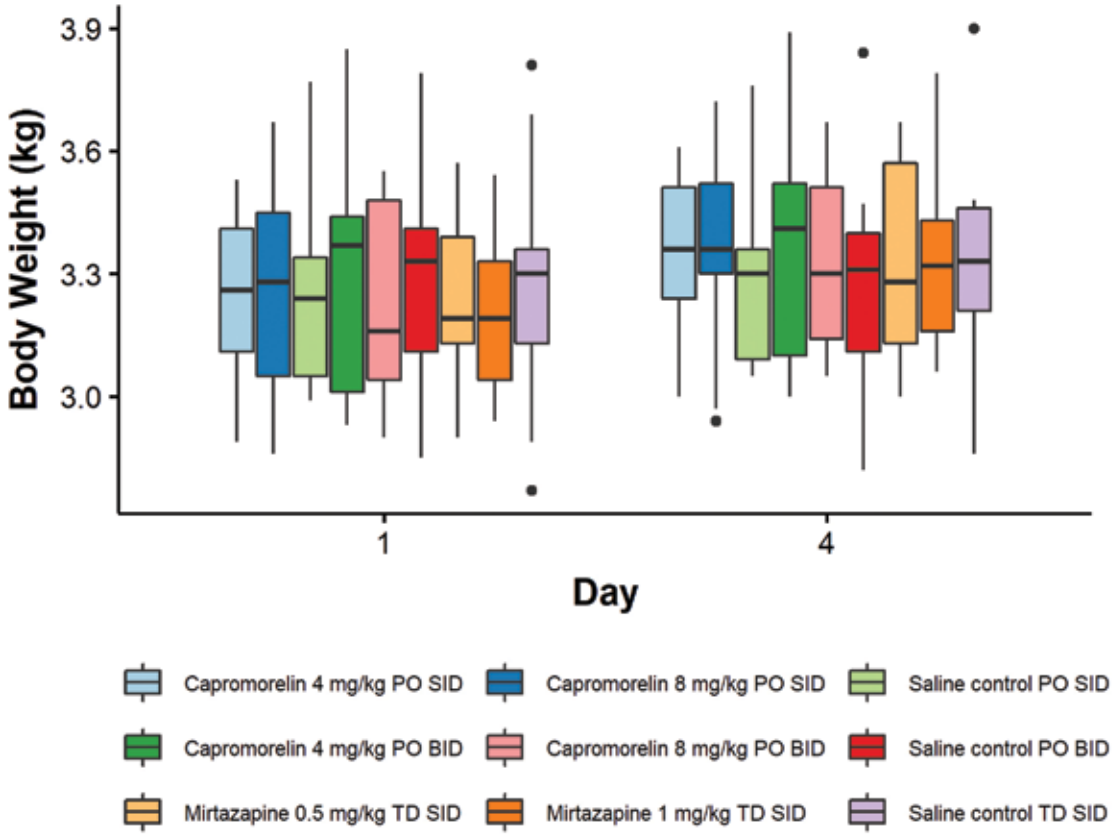

Figure 4. Body weight in kilograms, in 9 male, NZW rabbits that received treatments (n = 9) on days 1 to 3 in boxplots representing the median and interquartile range (IQR) with any individual point outside the IQR marked as dots. There was a significant increase in body weight in the mirtazapine 1 mg/kg (transdermal) TD SID as compared with saline control TD SID (P = 0.0019) from day 1 to 4.

Effects of mirtazapine on ear erythema and petechia and total ear score. The interaction between treatment and day was not statistically significant (P = 0.5899), but main effects of treatment and day were statistically significant (P = 0.0046 and P = 0.0251, respectively). Rabbits given mirtazapine 1.0 mg/kg TD SID had a significantly higher total ear score as compared with the saline control TD SID (P = 0.0035), and the total ear score was statistically greater on day 5 than on day one (P = 0.0248) (Figure 5). No significant differences were detected between other treatments. The erythema and petechia resolved within 3 to 5 d after discontinuing treatment. Despite the erythema and petechia, the ears did not appear to be painful or itchy.

Effects of capromorelin and mirtazapine on clinical presentation. No adverse treatment effects were observed in any of the rabbits at any time point, with the exception of the erythema and petechia of the ears in the transdermal mirtazapine groups.

Study Two: Effects of capromorelin and mirtazapine in postoperative NZW rabbits. ***Effects of capromorelin and mirtazapine on feed consumption, fecal output, and body weight.*** No significant differences were detected in feed consumption (Figure 6), fecal output (Figure 7), or body weight (Figure 8) between treatments (capromorelin 8 mg/kg PO BID, mirtazapine 1 mg/kg TD SID, saline control PO BID) at any time point.

Discussion

These studies indicate that appetite stimulants capromorelin and mirtazapine increase feed intake in healthy NZW male rabbits and should be considered as a potential treatment for rabbits with inappetence.

Inappetence is a welfare concern in animals and is especially problematic in rabbits, as inappetence can lead to GI stasis and death in this species.[19] Numerous studies have explored potential treatments for opioid-induced GI stasis and inappetence in rabbits, including the use of GI motility agents and peripheral opioid antagonists.[9,13] However, at the dosages used in those studies, cisapride and methylnaltrexone did not ameliorate the inappetence and prolonged GI transit times associated with buprenorphine administration.[9,13] We used an alternative approach to investigate ways to stimulate feed intake, prevent the onset of GI stasis and help to maintain a functioning gastrointestinal tract. Capromorelin and mirtazapine are commonly used appetite stimulants in dogs and cats.[4,5,14,18,21-26] Therefore, if they would be beneficial in rabbits, we performed 2 studies. In study one, we examined the effect of oral capromorelin and transdermal mirtazapine on feed intake, fecal output, and body weight in healthy NZW rabbits. Our results show that overall feed intake and fecal output were higher for all capromorelin and mirtazapine treatments as compared with the control group, except for fecal output in the capromorelin 4 mg/kg and 8 mg/kg PO SID groups (Figure 2 and 3). In addition, feed intake and fecal output were significantly higher in the mirtazapine treatments as compared with the capromorelin treatments (Figure 2 and 3). Both the ghrelin hormone, which capromorelin mimics, and histamine, which is targeted by mirtazapine, are highly conserved across species.[14,20] Therefore, the effects of these drugs on appetite in rabbits were unsurprisingly similar to the effects seen in dogs and cats.[16,21] These results suggest that oral capromorelin at 4 or 8 mg/kg SID or BID

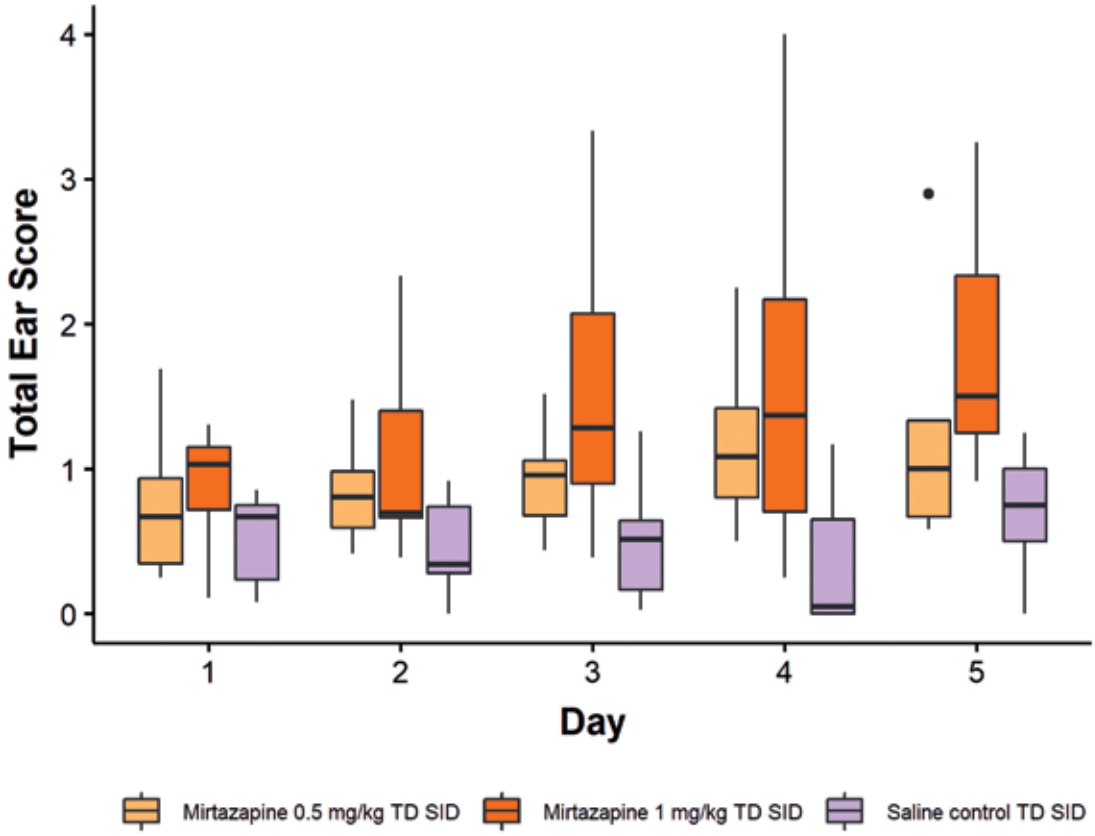

Figure 5. Total ear score, in 9 male, NZW rabbits that received treatments (n = 9) on days 1 to 3 in boxplots representing the median and interquartile range (IQR) with any individual point outside the IQR marked as dots. There was a significant increase in total ear score in the mirtazapine 1.0 mg/kg transdermal (TD) SID as compared with the saline control TD SID (P = 0.0035) independent of the day, and the total ear score was significantly greater on day 5 than on day 1 (P = 0.0248).

and transdermal mirtazapine at 0.5 or 1 mg/kg SID can be included in the treatment plan for NZW rabbits experiencing inappetence.

Based on the promising results of study one, we investigated the benefits of capromorelin and mirtazapine for treatment of postoperative inappetence in NZW rabbits. In study 2, we performed preadoption castration on the rabbits used in study one and administered our standard postoperative medications, the opioid analgesic buprenorphine and the nonsteroidal anti-inflammatory agent meloxicam. Surgical stress and opioid administration reduce feed intake; this reduction can lead to GI stasis in NZW rabbits.[9,13,17,20] However, significant increases in feed intake fecal output were not observed in rabbits receiving mirtazapine as compared with rabbits that received capromorelin and control treatments. Nonetheless, the effects in healthy rabbits suggest that mirtazapine may benefit rabbits (Figures 6 and 7).[18] The lack of statistical significance could be due to low sample size and the inability to perform a complete crossover study, as rabbits could only be castrated once. In addition, 2 rabbits were removed from study 2 due to surgical complications. Additional studies with more robust animal numbers are needed to fully assess transdermal mirtazapine as a treatment for opioid- and surgery-induced inappetence and GI stasis.

In general, healthy rabbits that received transdermal mirtazapine had greater feed intake and fecal output as compared with the rabbits in the oral capromorelin treatment groups (Figure 2 and 3). A possible explanation for mirtazapine's superior ability to increase feed intake and fecal output is the route of administration. Mirtazapine is available in both oral and transdermal formulations. We used the transdermal formulation, as it is easily applied to the inner pinnae of the ear. In addition, a recently published article investigating the effects of oral mirtazapine at a dose of 1 mg/kg or 3 mg/kg PO SID did not find an increase in feed intake.[15] We believe that our results differ from this recently published article due to differences in route of administration and dosages. Further, for transdermal application, rabbits do not require restraint for administration, resulting in less handling stress, which could have contributed to differences in the outcome of our study and the previous one. In addition, rabbits received the full dose of the treatment during the transdermal application. Conversely, capromorelin is only available as an oral formulation and therefore required manual restraint for treatment administration, resulting in handling stress. Furthermore, the rabbits seemed to dislike the taste of capromorelin and would actively avoid the treatment, refuse to swallow it, or spit it out. Oral gavage of capromorelin is possible but is less applicable to a clinical or at-home setting and was not used as a route of administration in our study. The longer half-life of mirtazapine as compared with capromorelin could also impact feed intake and fecal output. The half-life of mirtazapine is reported to be up to 26.8 h in cats while the half-life of capromorelin is 1.2 h in dogs.[4,14,21,25] The long half-life of mirtazapine may benefit rabbits, as they eat approximately 30 times per day due to a high energy demand and reliance on indigestible fiber for peristalsis.[19] Both the transdermal route

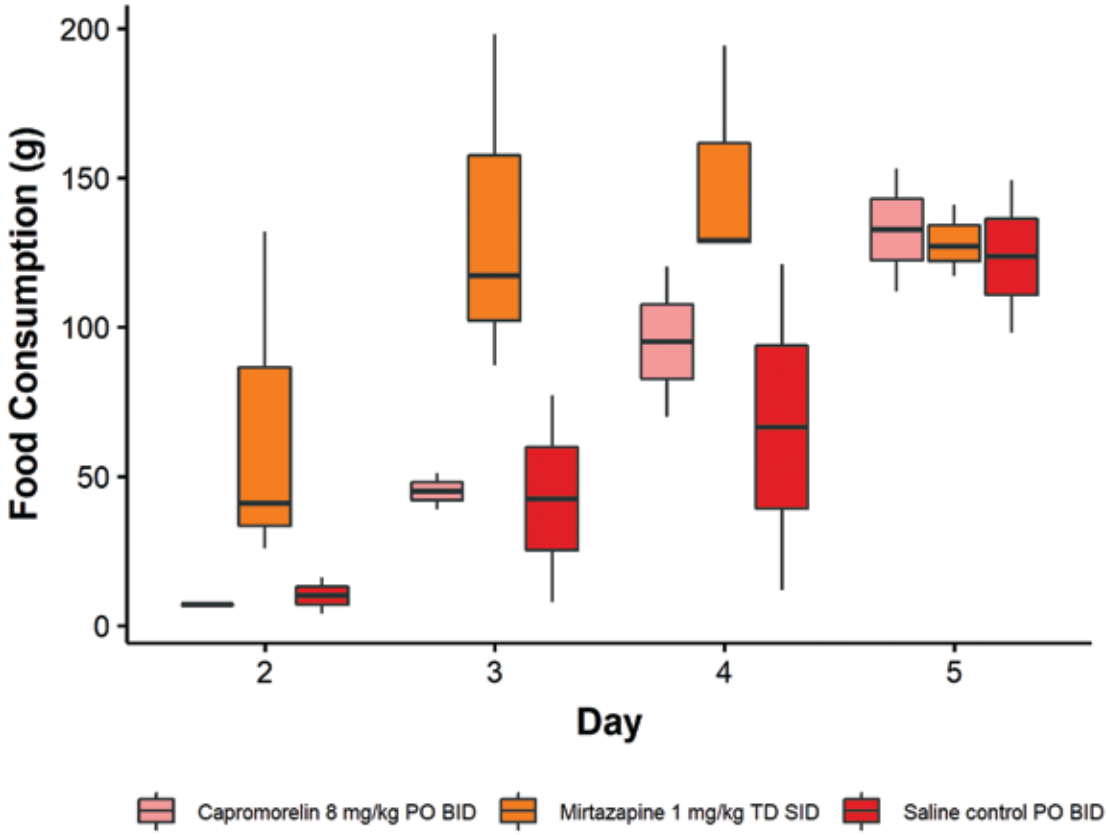

Figure 6. Feed intake in grams, as defined by amount consumed over 24 h, in 7 male, NZW rabbits after castration that received treatments capromorelin 8 mg/kg PO BID (n = 2) mirtazapine 1 mg/kg transdermal (TD) SID (n = 3), and saline control PO BID (n = 2) on days 1 to 3 in boxplots representing the median and interquartile range (IQR) with any individual point outside the IQR marked as dots. No significant effects were detected between the treatments at any time point. On day 2, rabbits receiving capromorelin 8 mg/kg PO BID ate the same amount and therefore the data appear as a single line.

of administration and the longer half-life of mirtazapine could have increased the efficacy of mirtazapine as compared with capromorelin in terms of feed intake and fecal output in our study. Additional studies are warranted to measure the half-life of these drugs in NZW rabbits.

We observed transient erythema and petechia at the site of mirtazapine administration on the inner pinnae of the ears. Mirtazapine 1 mg/kg TD SID caused a significantly higher ear score as compared with the saline control TD SID treatment (Figure 5). Erythema is one of the most common side effects of transdermal mirtazapine noted in cats.[14] No clinical interventions or treatments were warranted due to the topical irritation in our study and no clinical signs of pain were observed. The erythema and petechiae that lead to the increased ear scores were transient and resolved within 3 to 5 d after discontinuing treatment. The rabbits receiving the lower dose of mirtazapine (0.5 mg/kg TD SID) also had erythema and petechia of the pinnae, although the total ear score was not significantly higher than that associated with the saline control TD SID treatment. The benefits of appetite promotion in rabbits outweigh the transient topical side effects and should not preclude the use of topical mirtazapine. We recommend alternating use of the ears to minimize irritation and wearing disposable gloves to apply the treatment, as recommended by the manufacturer.

A surprising result of our study was the lack of significant difference in feed intake and fecal output between SID and BID treatment with capromorelin. We speculated that BID dosing increase appetite in rabbits as compared with SID administration due to the drug's short half-life. A greater frequency of administration could provide more consistent levels of the appetite stimulant and have longer-acting affects. However, numerous potential reasons could account for the failure of a higher treatment frequency to produce a larger change in appetite. The need to handle the rabbits for oral administration and the unpalatable taste of the capromorelin could have resulted in stress and dose inaccuracy sufficient to affect feed intake. The timing of the dosing could also have affected feed intake. Rabbits are a crepuscular species and are most active at dawn and dusk.[7] The first dose of capromorelin was administered in the morning but after dawn, and the second dose was administered in the afternoon, before dusk. Capromorelin may have a greater effect if administered closer to the rabbit's natural feeding times. Capromorelin in general may be the most efficacious when administered once a day, as is done in dogs and cats. Moreover, the high and low doses of both capromorelin and mirtazapine had no significantly different effects on feed intake, fecal output, or body weight. We suspect that the lower doses of both drugs were closer to the ideal dose of appetite

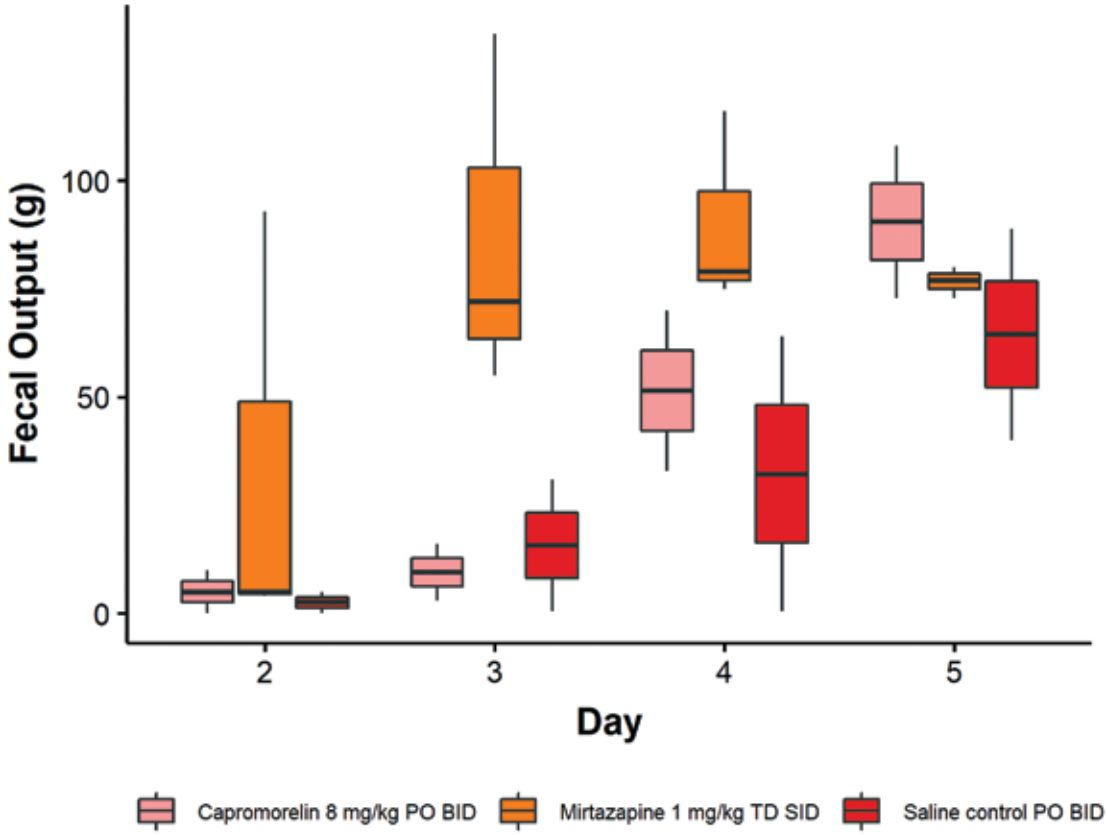

Figure 7. Fecal output in grams, as defined by amount defecated over 24 h, in 7 male, NZW rabbits after castration that received treatments capromorelin 8 mg/kg PO BID (n = 2) mirtazapine 1 mg/kg transdermal (TD) SID (n = 3), and saline control PO BID (n = 2) on days 1 to 3 in boxplots representing the median and interquartile range (IQR) with any individual point outside the IQR marked as dots. No significant effects were detected between the treatments at any time point.

stimulant in rabbits. Additional studies should be performed to determine the best dose of these drugs in this breed and species. Lastly, we saw no indication that treatments affected cecotroph production or consumption.

Multiple methods can be used to quantify appetite in rabbits. In our study, the primary parameter used was feed intake. We also measured fecal output and body weight, as these are correlated with changes in feed intake and can provide supporting evidence of increased appetite. Rabbits were housed on slotted floors with a tray to catch urine and feces underneath the cage. A fresh paper liner was placed in the tray daily to absorb urine and facilitate fecal collection. Despite the paper liner, we observed that urine and feces mixed, which could have changed the moisture content and weight of the feces. Therefore, fecal output was less consistent than the feed intake in terms of accurate sample collection. To avoid this complication in future studies, they should be conducted in caging systems designed to separate feces and urine.

Future research on the use of oral capromorelin and transdermal mirtazapine in rabbits may include toxicity, pharmacodynamics, and pharmacokinetics studies; clinical trials with client owned rabbits could be used to evaluate the use in different breeds and ages of rabbits. We used only male rabbits in our studies to limit hormonal interference in data collection, modeling our experiment after 2 previously published studies investigating GI transmit time in rabbits.[9,13] Future studies could assess the effects of these 2 appetite stimulants in female rabbits.

Our results show that overall, oral capromorelin and transdermal mirtazapine increased feed intake and fecal output in healthy NZW rabbits as compared with control treatment, and mirtazapine also increased body weight as compared with the control. Increases in these 3 parameters were used as evidence of increased appetite in this study. Both capromorelin and mirtazapine were well tolerated in rabbits. Mirtazapine was easier to administer accurately, but it caused transient erythema and petechia to the inner pinnae. If using capromorelin, we recommend a dose of 4 mg/kg SID PO to decrease handling stress and administration intolerance, as we saw no significant difference between high and low doses of capromorelin, or between SID and BID administration in terms of feed intake, fecal output, and weight gain. We recommend the use of transdermal mirtazapine over capromorelin as the former is easier to administer, with our recommendation of using mirtazapine at 0.5 to 1 mg/kg SID. Mirtazapine can be administered using a 1 mL syringe for accurate measuring and should be applied to the inner pinna, alternating the treated ear with each treatment. In conclusion, this is the first study to evaluate the effects of appetite stimulants capromorelin and mirtazapine in rabbits. The results support the use of transdermal mirtazapine as an adjunctive treatment of inappetence in rabbits.

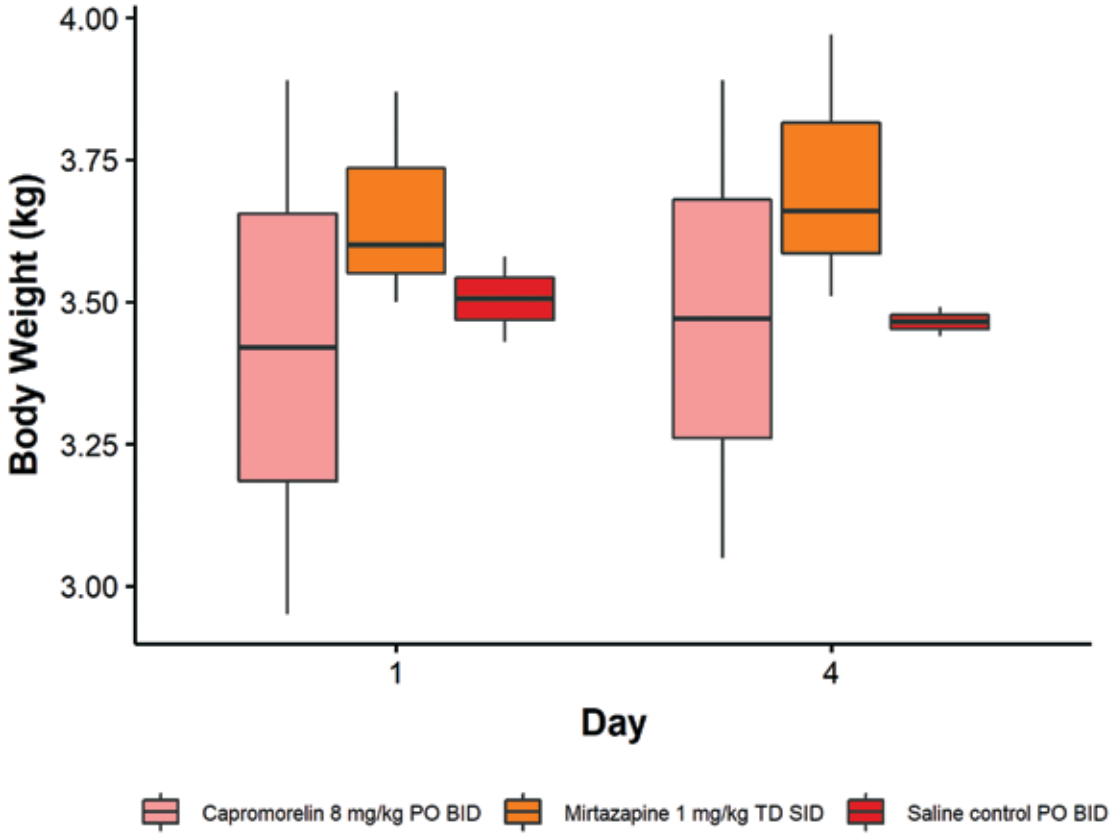

Figure 8. Body weight in kilograms, as defined by amount defecated over 24 h, in 7 male, NZW rabbits after castration that received treatments capromorelin 8 mg/kg PO BID (n = 2) mirtazapine 1 mg/kg transdermal (TD) SID (n = 3), and saline control PO BID (n = 2) on days 1 to 3 in boxplots representing the median and interquartile range (IQR) with any individual point outside the IQR marked as dots. No significant effects were detected between the treatments at any time point.

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