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Effects of Buprenorphine and Carprofen on Appetite in New Zealand White Rabbits (*Oryctolagus cuniculus*)

Zoe Y Hsi,[1,2,*] Jacob H Theil,[2] Betty W Ma,[2] Rhonda S Oates[1,2]

Rabbits are especially susceptible to adverse effects related to surgery, which can lead to inappetence and gastrointestinal (GI) stasis. However, these adverse effects may be related to discomfort from the procedure, anesthesia, the analgesics used, and the stress of restraint for analgesic administration. Opioid and NSAID analgesics which are frequently used in rabbits, can contribute to these adverse effects. This study compared the clinical GI side effects of buprenorphine and carprofen to saline controls in New Zealand White rabbits after a nonsurgical anesthetic event. Nine rabbits (3 females and 6 males, aged 8 to 20 mo) were randomly rotated through 5 treatment groups with a 7-d washout period between treatments: anesthesia control (no treatment), buprenorphine (0.05 mg/kg SC every 12 h for 72 h), carprofen (5 mg/kg SC every 24 h for 72 h), twice daily saline control (equivalent volume to buprenorphine SC every 12 h for 72 h), and once daily saline control (equivalent volume to carprofen SC every 24 h for 72 h). All rabbits were anesthetized 5 times and received initial treatments on the day of anesthesia. Generalized linear mixed models were used to assess food intake, water intake, and fecal output score for 7 days after anesthesia. Analysis showed that buprenorphine-treated rabbits had a significant 4-d decrease in food intake and a 3-d decrease in fecal output score compared with baseline. None of the other treatment groups showed any changes in food intake or fecal output score compared with baseline. These findings demonstrate that in the absence of pain, buprenorphine significantly depresses food intake in rabbits and that restraint and injections have minimal effect on food intake despite the possibility of increased stress.

Abbreviations and Acronyms: GI, gastrointestinal

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Introduction

Surgical and other painful procedures performed on animals in biomedical research require the use of analgesic therapy to maintain acceptable animal welfare standards. While the body of literature on veterinary analgesics is growing, the effects of analgesic regimens on basic physiologic parameters are unknown for many veterinary species, breeds, and strains due to their unique physiology, anatomy, and behavior.

For rabbits (*Oryctolagus cuniculus*), opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) are cornerstones of analgesic therapy in both clinical and research settings, particularly for alleviation of postsurgical pain. Common opioid choices include buprenorphine, butorphanol, morphine, and fentanyl, with buprenorphine appearing to be used most frequently.[13] Buprenorphine, a partial mu receptor agonist, is effective at controlling postsurgical pain in several mammalian species.[18,35,41,45] Nonsteroidal anti-inflammatory drugs (NSAIDs) such as carprofen and meloxicam are also used routinely for their anti-inflammatory, analgesic, and antipyretic properties.[34] However, both opioids and NSAIDs are associated with gastrointestinal (GI) side effects that can complicate the assessment of analgesic efficacy and postsurgical recovery. GI side effects are particularly important in rabbits, a species for which inappetence and reduced GI motility can rapidly become life-threatening if untreated.[26] For opioids, potential adverse effects include reduced peristalsis and motility of the GI tract.[20] Side effects of NSAIDs include GI irritation and ulceration, with increased risk from chronic administration, high dosages, and certain classes of NSAIDs.[34]

In addition to the drugs themselves, the dosing schedule should be considered when selecting an analgesic, as handling, restraint, and injections can be stressful to animals. Rodents develop increased heart rate, blood pressure, and blood corticosterone in response to such stressors[7,33,38] and stress hyperglycemia has been observed in rabbits.[24] The effects of stress on the GI system are numerous. Stimulation of the sympathetic nervous system decreases activity of the GI tract and reduces gut motility.[25] Acute and chronic stress can alter eating behavior, leading to both increased[29,32] and decreased food intake.[36,43] While buprenorphine is fairly long-lasting and is typically administered every 12 h, some evidence suggests that its analgesic effects may last for only 8 to 10 h and that more frequent administration is necessary for full pain coverage.[4] However, more frequent dosing may not be practical in the research setting, and may also increase stress levels in a sensitive species. In comparison, carprofen requires a less frequent dosing regimen, and in rabbits is typically administered once or twice daily depending on the dose.[8] However, this dosing interval is based on extrapolation of pharmacokinetic knowledge from other species.

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[1]School of Veterinary Medicine, University of California, Davis, Davis, California,
[2]Campus Veterinary Services, University of California, Davis, Davis, California
[*]Corresponding author. Email: zoe.hsi@jax.org

The objective of this study was to compare the GI side effects of buprenorphine and carprofen with saline controls in New Zealand White rabbits after a nonsurgical anesthetic event. Investigating these differences provides a better understanding of whether decreased appetite and GI side effects are a direct result of the analgesic used or related to handling stress and anesthesia. We hypothesized that adverse GI effects and more injections per day would be positively related, and that these effects would exacerbate the GI signs associated with buprenorphine and carprofen. This understanding would inform analgesic decisions for rabbit pain management after surgical and other painful procedures.

Materials and Methods

Animals. This study was approved by the University of California, Davis IACUC and was conducted in compliance with the *Animal Welfare Act*,[2] *Animal Welfare Regulations*,[3] and *Guide for the Care and Use of Laboratory Animals*.[27]

The study comprised 9 healthy New Zealand White rabbits (3 female and 6 male, aged 8 to 20 mo, approximate weight 3.0 to 4.0 kg), which were used based on availability. Treatment group sizes were based on veterinary estimates of food intake and calculated assuming an effect size of 50 g, a standard deviation of 35 g, a level of significance of $P = 0.05$, and a power of $1\text{-}\beta = 0.8$.[28] All rabbits had been received from Charles River Laboratories (Oxford, MI) and were transferred from a noninvasive protocol for teaching handling skills and physical examinations to veterinary students once to twice yearly. Rabbits were transferred back to the training protocol after study completion. No rabbits had been used for several months prior to this study. All rabbits were certified SPF by the vendor for the following pathogens: all ecto- and endoparasites, reovirus, lymphocytic choriomeningitis virus, parainfluenza virus, rotavirus, rabbit hemorrhagic disease virus, *Bordetella bronchiseptica*, *Helicobacter* spp., *Lawsonia* sp., *Pasteurella multocida*, *Pasteurella* spp., *Pseudomonas aeruginosa*, *Salmonella* spp., *Treponema*, Tyzzer disease, *Filobacterium rodentium*, *Eimeria stiedae*, and *Encephalitozoon cuniculi*. Rabbits were housed individually in stainless steel cages ($26 \times 23 \times 16$ in. [$66 \times 58 \times 41$ cm]) with perforated floors suspended above a collection tray. Each cage contained a piece of PVC pipe for enrichment. Rabbits were fed commercial rabbit diet (Laboratory Rabbit Diet HF; LabDiet; St. Louis, MO) with access to water in plastic sipper bottles. Standard husbandry protocol includes daily hay cubes for supplemental feeding, but hay cubes were not given during this study to allow for more accurate food intake measurements. The housing room was maintained at 61 to 72 °F (16 to 22 °C) at 30% to 70% humidity, on a 12:12-h light cycle (lights on at 0600 and off at 1800). All rabbits were acclimated to environmental conditions for 1 wk prior to the study.

Baseline parameter recording. Prior to the study, all rabbits were weighed and underwent a physical examination to ensure their health. Rabbits received one liter of water in sipper bottles and exactly 339 g (3 times their normal ration) of commercial rabbit diet daily to allow ad libitum feeding. At 24 h after rabbits received food and water, the following were measured: food intake (measured in grams), water intake (measured in milliliters), fecal output score, fecal quality (subjective), and urine output (subjective). Values were not corrected for food or water spillage. Baseline parameters were recorded on day 0 of the study and were repeated for every week of treatment. Values were recorded between 0700 and 0800 each day by a single individual who was aware of the treatment.

Body weight was measured in kg kilograms using an infant scale (KEDSUM; Hong Kong Pennybuying Tech; Hong Kong). Physical examination parameters included temperature, heart rate, respiratory rate, mucous membrane color, capillary refill time, hydration assessment, auscultation, and abdominal palpation. Food intake was measured using a gram scale to weigh the food remaining after 24 h of consumption and subtracting the result from the initial 339 g of commercial diet. The feeders were then filled again with 339 g of commercial diet. Fecal output was assessed every 24 h by scoring each quadrant of the collection tray from 0 to 4 (0, less than 25 fecal pellets; 1, 25 to 50 fecal pellets; 2, 51 to 75 fecal pellets; 3, 76 to 100 fecal pellets; 4, greater than 100 fecal pellets), then averaging the sum. Fecal quality assessment included visible assessment for presence of cecotropes, soft feces, desiccated feces, small diameter feces, and other abnormalities. Amount of water consumed per 24 h was measured by the observer based on volume markings on the plastic sipper bottle. A subjective assessment of urine output was made based on the presence or absence of urine in the collection tray.

Treatment groups. A randomized crossover design was used to evaluate clinical gastrointestinal side effects in 5 treatment groups: 1) anesthesia control (no treatment), 2) buprenorphine (0.05 mg/kg SC every 12 h for 72 h; Par Pharmaceutical, Chestnut Ridge, NY), 3) carprofen (5 mg/kg SC every 24 h for 72 h; Putney, Portland, ME), 4) twice daily saline (equivalent volume to buprenorphine dosage SC every 12 h for 72 h; 0.9% sodium chloride, Hospira, Lake Forest, IL), and 5) once daily saline (equivalent volume to carprofen dosage SC every 24 h for 72 h; 0.9% sodium chloride). Each study period lasted 8 d (days 0 to 8). Rabbits were anesthetized on day 0 of each study period (5 times total) and treatments were administered on days 0 to 2 at 0700 (for once daily treatments) and 0700 and 1900 (for twice daily treatments) each day. Rabbits were randomly assigned to treatment groups each week until every rabbit had been enrolled in each of the 5 treatment groups. After day 8 of each study period, rabbits underwent a washout period of at least 7 d before random enrollment in a different treatment group. Due to supply issues, on day 2 of the 4th treatment period, we switched to different manufacturer of carprofen (Rimadyl, Zoetis, Parsippany, NJ), which was administered at the same concentration and dose. Dosages were based on the high end of published recommendations.[8] Subcutaneous injections were administered in the scruff region and performed by a single individual who was aware of the treatment. Rabbits were restrained and injected in their cages with a 22-gauge needle.

Anesthesia. Rabbits were sedated with ketamine (5 mg/kg IM; Zetamine, VetOne, Boise, ID) and midazolam (0.2 mg/kg IM; West-Ward Pharmaceuticals; Eatontown, NJ). The 2 drugs were combined into one syringe and injected into the epaxial muscles by a single individual. Fifteen minutes after sedation, rabbits were administered isoflurane anesthesia (1.5%; Fluriso, VetOne, Boise, ID) mixed with oxygen (1.5 L/min) via face mask and placed in left lateral recumbency. Measured parameters included heart rate, respiratory rate, and rectal temperature. Anesthesia was maintained for 20 min. The first treatment dose was administered immediately after turning off the anesthetic gas. Rabbits were allowed to recover in a warmed environment until they resumed a sternal position. Once fully awake, rabbits were returned to their home cages and provided 339 g of commercial diet. Anesthesia was performed between 0800 and 1100 on day 0 of each study period after baseline parameter recording.

Daily parameter recording. Each rabbit was monitored for 7 d after the anesthetic event for each study period. Daily parameters measured were the same as described above for

baseline parameters. To mimic normal clinical intervention, any rabbit that exhibited inappetence (ingestion of less than 50 g of commercial diet) and/or scant to absent fecal output for 2 consecutive days received a physical examination and was supplemented daily with a handful of timothy hay and a mixture of applesauce, plain canned pumpkin, probiotic powder (Probios, Vets Plus, Menomonie, WI), and Critical Care Apple Banana (Oxbow, Omaha, NE). Supplemental provisions were removed after 3 consecutive days of good appetite (ingestion of greater than 50 g of commercial diet) and were not included in food intake calculations.

Statistical analysis. Treatment group enrollment randomization was performed with Excel 2019 (Microsoft, Redmond, WA). Data were entered into a spreadsheet (Excel 2019) and exported into SPSS Statistics for Windows (version 27, IBM, Armonk, NY) for analyses. To test for differences in food intake, data were analyzed by using a generalized linear mixed repeated-measures model. The dependent variable was daily grams of food consumed with the subject variable being rabbit and repeated measures being treatment and study day. Factors for analysis included treatment, study day, and study week. The 2-way interaction between treatment and study day was added to the analysis due to model relevance and improved model fit which was assessed via Akaike Information Criterion (AIC). Factors such as sex and age were not included in the analyses due to the study's limited sample size. Post hoc analysis was only performed on the interaction variable of treatment and study day due to study relevance. Contrasts of this interaction were performed using pairwise comparisons and a Bonferroni-adjusted significance of 0.05. Similar analysis was performed for fecal output score. For water intake, a similar model was used but with the interaction variable removed due to lack of statistical significance and model fit. For water intake, post hoc analysis was performed via pairwise comparisons of study day and treatment using a Bonferroni-adjusted significance of 0.05 as well. Post hoc analysis was not performed for study week due to lack of relevance to the study but was maintained in the models to control for time related effects. Descriptive statistics were expressed as mean +/- SEM.

Results

Food intake. For food intake (n = 9 for each treatment group), treatment (F = 41.227, P < 0.0005), study day (F = 6.158, P < 0.0005), week (F = 7.190, P < 0.0005), and the interaction between treatment and study day (F = 3.611, P < 0.0005) were significant predictors of food intake Figure 1A. Post hoc analysis showed that of all treatment groups, only the buprenorphine treatment showed significant reductions in food intake on day 1 (t = 5.469, P < 0.0005) after anesthesia as compared with before anesthesia. Reduced food intake also occurred on days 2 (t = 11.335, P < 0.0005), 3 (t = 9.507, P < 0.0005), and 4 (t = 4.097, P = 0.001) before returning to baseline on day 5 (t = 2.609, P = 0.113). The majority of buprenorphine-treated rabbits (6 of 9) received supplemental provisions on day 2 due to 2 consecutive days of poor appetite. Provisions were removed on day 5 (n = 3), day 6 (n = 2), or day 7 (n = 1) after 3 consecutive days of good appetite. Values for food intake by treatment over the 7-d period are shown in Figure 1A.

Rabbits that received buprenorphine had significantly less food intake than all other treatment groups on day 1 through day 4. On day 1, buprenorphine-treated rabbits had significantly less intake did than control (t = 4.030, P = 0.001), carprofen (t = 4.531, P < 0.0005), twice daily saline (t = 3.564, P = 0.003), and once daily saline-treated (t = 3.668, P = 0.002) rabbits.

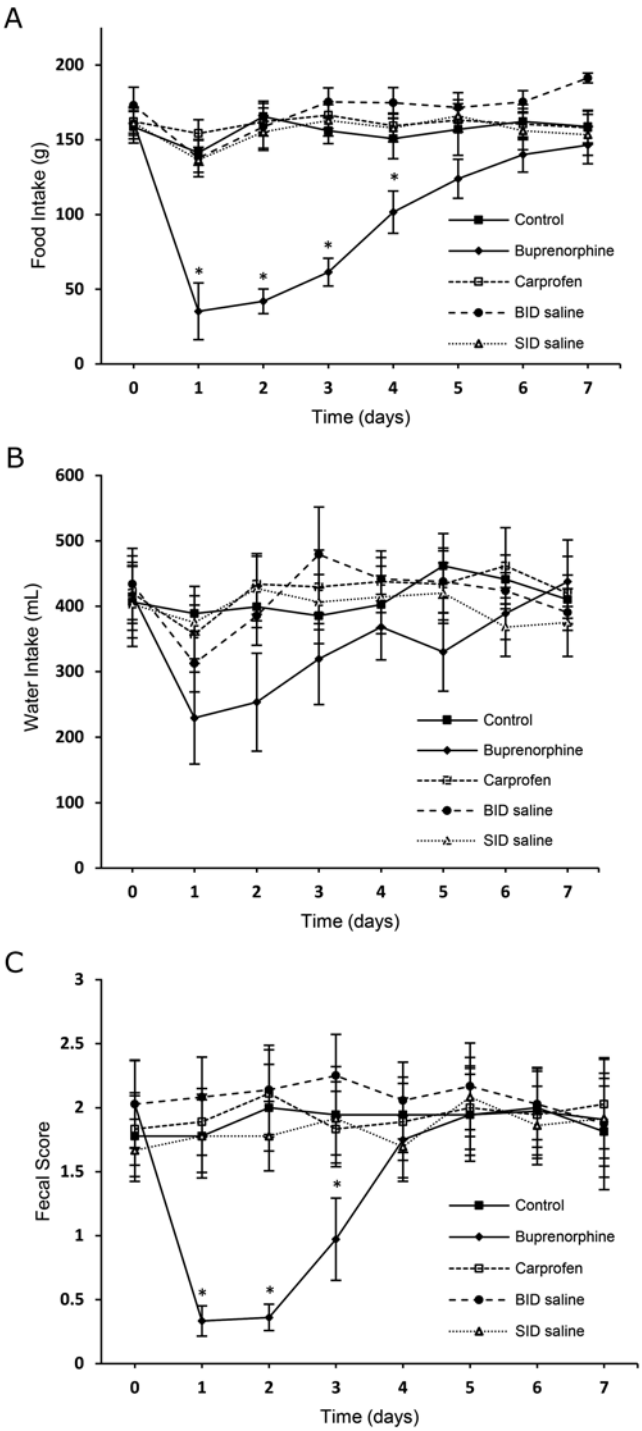

Figure 1. Means and standard error bars for food intake (A), water intake (B), and average fecal score (C) before (Day 0) and after (Day 1–7) anesthesia and treatment administration (*, significant difference compared with Day 0 as calculated by post hoc analysis of a generalized linear mixed model). Sample size for each treatment group was n = 9. Treatments were administered on Day 0–2 at 0700 (for once daily treatments) and 0700 and 1900 (for twice daily treatments) each day.

This reduction persisted through day 4 for control (t = 2.699, P = 0.051), carprofen (t = 3.552, P = 0.004), twice daily saline (t = 4.649, P < 0.0005), and once daily saline-treated (t = 3.344, P = 0.007) rabbits

Buprenorphine-treated rabbits ate significantly less than did twice daily saline treated rabbits through study day 7 (t = 4.858,

$P < 0.0005$). Control ($t = 2.787$, $P = 0.040$), carprofen ($t = 3.303$, $P = 0.009$), and once daily saline-treated ($t = 3.340$, $P = 0.008$) rabbits all also had significantly less food intake than twice daily saline-treated rabbit on study day 7.

Water intake. For water intake ($n = 9$ for each treatment group), treatment ($F = 3.503$, $P = 0.008$), study day ($F = 2.083$, $P = 0.045$), and study week ($F = 24.082$, $P < 0.0005$) were all significant predictors of water intake. Post hoc analysis showed significant reductions in water intake in rabbits receiving buprenorphine as compared with control ($t = 3.225$, $P < 0.014$) and twice daily saline-treated ($t = 3.024$, $P < 0.024$) rabbits. No other significant effects were detected. Values for water intake by treatment over the 7-d period are shown in Figure 1B.

Fecal output score. For fecal output score ($n = 9$ for each treatment group), treatment ($F = 6.661$, $P < 0.0005$), week ($F = 10.816$, $P < 0.0005$), and the interaction between treatment and study day ($F = 2.081$, $P = 0.001$) were significant predictors of fecal score. The values for fecal score by treatment over the 7-d period are shown in Figure 1C.

Post hoc analysis showed that of all treatment groups, only rabbits that received buprenorphine had significant reductions in fecal score on day 1 ($t = 4.708$, $P < 0.0005$) after anesthesia as compared with values before anesthesia. This reduction in fecal score persisted through days 2 ($t = 4.634$, $P < 0.0005$) and 3 ($t = 2.288$, $P = 0.023$) and returned to baseline on day 4 ($t = 0.571$, $P = 0.569$).

Rabbits that received buprenorphine had significantly lower fecal scores than all other treatment groups from day 1 through day 3. On day 1, buprenorphine-treated rabbits had significantly lower fecal scores than control ($t = 4.902$, $P < 0.001$), carprofen ($t = 5.705$, $P < 0.0005$), twice daily saline ($t = 5.930$, $P < 0.0005$), and once daily saline-treated ($t = 4.215$, $P < 0.0005$) rabbits. Reductions persisted through day 3 for control ($t = 2.370$, $P = 0.018$), carprofen ($t = 1.998$, $P = 0.047$), twice daily saline ($t = 3.224$, $P = 0.001$), and once daily saline-treated ($t = 2.037$, $P = 0.047$) rabbits. All other fecal score comparisons were not statistically significant.

Based on visual assessment, buprenorphine-treated rabbits subjectively had more fecal quality abnormalities than did compared with other treatment groups. On day 1 through day 2, the majority of fecal pellets from buprenorphine-treated rabbits were desiccated and irregularly shaped. By day 3 only minimal abnormalities were noted, and by day 4 all feces were observed to be of normal quality. All remaining treatment groups had moist, spherical fecal pellets on all study days.

Urine output. The majority of buprenorphine-treated rabbits (6 of 9) had no urine output on day 1, and 1 of 9 rabbits had no urine output on day 2. By day 3, all buprenorphine-treated rabbits produced urine. All rabbits in the other treatment groups produced urine throughout the entire study period.

Discussion

After surgery in rabbits, discomfort from the procedure, the anesthetic event, the analgesic drug used, and the stress of handling can all contribute to a poor appetite. Consistent with the current literature, results from the current study suggest that buprenorphine administration significantly contributes to the observed adverse GI signs. Buprenorphine has been associated with decreased GI motility in horses[15] and rats,[14] pica and gastric distention in rats,[10] self-limiting reduction in food intake and fecal output in chinchillas,[19] and weight loss and reduced food intake in postoperative mice.[22]

Recent literature suggests that the reduction in GI motility caused by buprenorphine in rabbits does not require clinical intervention. For example, a single dose of intramuscular buprenorphine at 0.1 mg/kg did not appear to affect GI motility in healthy New Zealand White rabbits.[17] Healthy rabbits given multiple doses of buprenorphine at 0.05 mg/kg SC had reduced food and water intake, decreased fecal output, and prolonged GI transit time, but did not require medical intervention.[31] After surgery, buprenorphine has been associated with mildly reduced food intake and fecal output without development of overt GI stasis in several studies in rabbits.[1,12,37] Because pain can manifest as anorexia in rabbits,[4] the current study did not include surgery so that any changes in adverse effects could be attributed to the drug itself. Our results show that in the absence of surgical pain, buprenorphine is associated with a significant reduction in food intake that normalizes after drug administration ends. To balance the effects of buprenorphine on appetite and pain control, more research is needed to investigate the effects of alternative dosing durations, dosages, and dosing frequencies.

Despite common use, little published information is available on the potential side effects of NSAIDs in rabbits. In a study investigating the pharmacokinetics and safety of oral meloxicam in rabbits, no overt adverse effects were noted after daily administration for 5 d.[42] After ovariohysterectomy, meloxicam-treated rabbits had mild decreases in food intake and fecal output that were comparable to those of buprenorphine-treated rabbits.[12] In other species, GI adverse effects such as ulceration typically occur more frequently with chronic use.[30] However, a single dose of ketoprofen can cause acute GI bleeding and ulceration in rats.[39] In this study we did not expect carprofen to significantly affect the GI system due to the short duration of treatment. Our data showed that carprofen had little effect on food intake as compared with baseline, with no carprofen-treated rabbits requiring dietary supplementation. As with buprenorphine, more research is needed to assess potential adverse effects associated with chronic use and higher dosages of this class of drugs. Multimodal analgesia (such as a combination of an opioid and an NSAID) was not investigated in this study, and further research is warranted to assess the effects of multiple drug interactions on GI health.

The effects of ketamine/midazolam sedation followed by isoflurane anesthesia on food intake have not been published in rabbits; most literature focuses on rodents. In one study, chronic administration of ketamine in rats increased body weight and consumption of sweet food,[21] whereas another study showed weight loss.[44] Single doses of midazolam and other benzodiazepines can stimulate appetite in rodents and humans.[5] Several studies in rodents found no change in food intake after isoflurane anesthesia.[5,9,46] Only buprenorphine-treated rabbits had statistically significant decreases in food intake and fecal output compared with baseline, indicating that the overall effects of sedation and anesthesia were minimal. This result was likely due to the low drug dosages and short anesthetic events, and more research is indicated to assess the effects of these drugs at different dosages and durations.

To test for the effects of handling stress on rabbits, we compared the effects of injected analgesics to those of injected saline. Stress affects GI health in many species, and rabbits are particularly sensitive. Rabbits can develop anorexia and GI stasis from stress of many etiologies,[16] and many rabbits display fear-type behaviors when lifted.[6] As a result, we hypothesized that handling rabbits twice a day for injection would decrease appetite and fecal output as compared with injections once a day or not at all. Saline injections had no statistically significant effects on food intake as compared with

baseline. Although longer dosing intervals are advantageous for time and labor purposes, the effect of increased handling for injections appears minimal. The importance of this finding is that buprenorphine's duration of action may be shorter than the reported 12 h[4], making 3 daily injections potentially necessary for more painful procedures. However, the relationship between stress and eating behavior is variable. Acute and chronic stress can cause either increased or decreased eating in several species, including humans.[29,32,47] We did not measure other markers of stress (such as serum cortisol), and because this cohort of rabbits was previously used for teaching purposes, they were well acclimated to handling prior to the current study. Frequent handling can promote compliance and reduce stress in rabbits and rodents, improving both animal welfare and worker safety and satisfaction.[11,23,40] Further research is needed to assess the effects of handling and injection on food intake in rabbits that are not accustomed to handling as compared with those that are well-accustomed to handling.

A limitation of this study was the demographics of the study population. Rabbits were sourced from a noninvasive training protocol and enrolled in the study based on availability. As a result, the sex ratio was unequal and the age range was broad. In addition, because we wanted to investigate clinical GI side effects unrelated to pain, the rabbits did not undergo surgery or any painful procedures. However, including surgery as a variable would more closely mimic a real-life scenario.

Analgesia should be implemented whenever pain is expected, such as after surgery. However, the adverse effects of analgesia must be considered when selecting drug type and dosages. In the absence of clinical pain and at the dosage used in this study, carprofen caused minimal change in food intake. In contrast, buprenorphine caused a significant reduction in food intake that quickly normalized once drug administration ceased. The effect of handling on food intake in frequently handled rabbits appears to be minimal, and greater frequency of injection would not be expected to significantly influence analgesic choice. Because buprenorphine at the dosage we used causes a significant reduction of food intake in rabbits, we recommend close monitoring of food intake and fecal output in buprenorphine-treated rabbits.

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