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Pharmacokinetics and efficacy of orally administered acetaminophen (paracetamol) in adult horses with experimentally induced endotoxemia

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Abstract

Background: Acetaminophen has been evaluated in horses for treatment of musculoskeletal pain but not as an antipyretic.

Objectives: To determine the pharmacokinetics and efficacy of acetaminophen compared to placebo and flunixin meglumine in adult horses with experimentally induced endotoxemia.

Animals: Eight university owned research horses with experimentally induced endotoxemia.

Methods: Randomized placebo controlled crossover study. Horses were treated with acetaminophen (30 mg/kg PO; APAP), flunixin meglumine (1.1 mg/kg, PO; FLU), and placebo (PO; PLAC) 2 hours after administration of LPS. Plasma APAP was analyzed via LC-MS/MS. Serial CBC, lactate, serum amyloid A, heart rate and rectal temperature were evaluated. Serum IL-1β, IL-6, IL-8, IL-10, and TNF-α were evaluated by an equine-specific multiplex assay.

Results: Mean maximum plasma APAP concentration was 13.97 ± 2.74 μg/mL within 0.6 ± 0.3 hour after administration. At 4 and 6 hours after treatment, both APAP ($P = <.001$, $P = .03$, respectively) and FLU ($P = .0045$ and $P < .001$, respectively) had a significantly greater decrease in rectal temperature compared to placebo. FLU caused greater heart rate reduction than APAP at 4 and 6 hours ($P = .004$ and $P = .04$), and PLAC at 4 hours ($P = .05$) after treatment.

Conclusions and Clinical Importance: The pharmacokinetics of acetaminophen in endotoxemic horses differ from those reported by previous studies in healthy horses.

Abbreviations: λ_z, terminal phase rate constant; APAP, acetaminophen; AUC, area under the plasma concentration versus time curve; Cl, clearance; C_{max}, maximum plasma concentration; COX, cyclooxygenase; F, bioavailability; FLU, flunixin meglumine; GEE, generalized estimating equation; HgB, hemoglobin; IL, interleukin; LC-MS/MS, liquid chromatography tandem mass spectrometry; LLOD, lower limit of detection; LLOQ, lower limit of quantification; LPS, lipopolysaccharide; NSAID, nonsteroidal antiinflammatory drug; PGE_2, prostaglandin E_2; PLAC, placebo; RBC, red blood cell; SAA, serum amyloid A; $T_{1/2}$, half-life of the terminal phase; T_{max}, time to maximum plasma concentration; TNF-α, tumor necrosis factor α; TXA_2, thromboxane A_2; V_d, volume of distribution; WBC, white blood cell.

Acetaminophen is an option for antipyresis in clinical cases, particularly when administration of traditional NSAIDs is contraindicated.

KEYWORDS
acetaminophen, fever, horse, pyrexia

1 | INTRODUCTION

The most utilized antipyretic drugs in equine practice are nonsteroidal antiinflammatory drugs (NSAIDs), particularly the nonselective cyclooxygenase (COX) inhibitors, such as flunixin meglumine and phenylbutazone, which act through inhibition of prostaglandin synthesis and release. Whereas nonselective COX inhibitors are effective as antipyretic drugs, they have a multitude of complications associated with prolonged or high-dose use, particularly in critically ill horses that might be dehydrated.[1-6] Until recently, there were no labeled antipyretic drugs available in horses. However, dipyrone IV (Zimeta Dechra Veterinary Products, Overland Park, Kansas) is approved for the control of fever in horses. Whereas formulations for oral administration of the nonselective COX inhibitors are readily available, there are no approved oral formulations of dipyrone for use in horses. Therefore, there is a clinical need for a safe, effective oral antipyretic drug in equine practice.

Acetaminophen (chemical name: N-acetyl-para-aminophenol or APAP, INN: paracetamol) is the most commonly recommended first line antipyretic drug in humans. In a study where human volunteers were infused with LPS, pretreatment with acetaminophen alleviated the clinical signs of fever response better than placebo or aspirin but did not change inflammatory cytokine expression.[7] Furthermore, acetaminophen alternated with the nonselective COX inhibitor ibuprofen has improved efficacy in the treatment of refractory fevers in children than either drug alone.[8] In a mouse model of LPS induced fever, acetaminophen mitigates the fever response through central suppression of prostaglandins, most critically complete suppression of the pyrogen PGE_2.[9,10] In veterinary species, acetaminophen is effective as an antipyretic in a LPS-induced fever model in swine, though it is less effective than ketoprofen.[11] Horses, in contrast to swine, possess a lower first pass effect for acetaminophen, which could allow for the drug to be more clinically effective.[12] In humans, the effective plasma concentration for antipyresis is 4.68 μg/mL, which is considerably lower than that for analgesia (9.98-25 μg/mL).[13-15]

In a previously published study, acetaminophen dosed at 30 mg/kg PO in healthy adult horses remained above the human therapeutic threshold for antipyresis for 8 hours after administration, however, terminal elimination kinetics for this dose were not determined.[16] The authors have utilized acetaminophen at 30 mg/kg PO in horses with fevers refractory to traditional nonsteroidal antiinflammatories with success, however, there have been no studies of the pharmacokinetics or efficacy of acetaminophen in horses with naturally occurring or experimentally induced fever. This study hypothesizes that acetaminophen will have superior antipyretic activity compared to placebo and equivalent antipyretic activity to flunixin meglumine in a low dose endotoxemia model in adult horses.

2 | MATERIALS AND METHODS

2.1 | Animals

This randomized placebo-controlled crossover experimental trial utilized 8 healthy adult horses between 8 and 15 years of age and with body weights ranging between 450 and 571 kg. The horses consisted of 5 geldings and 3 mares, and breeds included Thoroughbred (n = 5), Cleveland Bay (n = 1), American Paint Horse (n = 1), and Dutch Warmblood (n = 1). Horses were current on vaccinations and anthelmintic treatments, with no history of liver disease or dysfunction and were maintained as part of a university research herd. Horses were evaluated immediately before study initiation by physical exam, complete blood count, large animal chemistry panel, and serum amyloid A and were found to be healthy and free of any evidence of systemic inflammation. Horses were housed in individual, temperature controlled-box stalls during each 36-hour study period, to which they were acclimated 12 hours before study protocol initiation. They were fed grass hay and water ad libitum during the study period. During the 30 day washout periods between study periods, horses were maintained on local grass pasture and provided ad libitum water. This study protocol was approved by Virginia Polytechnic Institute and State University.

2.2 | Model induction

Twelve hours before each trial initiation, an IV catheter was placed in each jugular vein (MILA International, Inc., Erlanger, Kentucky). A low dose endotoxemia model was used to induce pyrexia.[17-19] Horses were weighed within 24 hours of each treatment day, and doses were calculated based on body weight. Lipopolysaccharide (LPS, E. Coli 055:b5 Sigma Aldrich, St. Louis, Missouri) was administered at 35 ng/kg IV in 500 mL of lactated Ringer's solution (LRS, Hospira Inc, Lake Forest, Illinois) over 30 minutes with an infusion pump through one of the jugular catheters.[20-22] To ensure the entire dose was administered, the fluid lines were flushed with 60 mL of LRS after the infusion. At that time, the LPS-dedicated IV catheter was removed and sample collection was performed from the IV catheter in the contralateral jugular vein. Each trial was separated by a 30-day washout period to minimize the risk of acclimation to LPS.[23]

2.3 | Drug administration

Horses were randomly assigned drug treatment order with a random number generator.[24] Horses were weighed within 24 hours of each treatment day, and doses were calculated based on current body weight. Two hours after LPS treatment, the horses received 1 of the 3 treatments. Acetaminophen (APAP; 500 mg caplet, Up&Up, Target Brands, Inc., Minneapolis, Minnesota) was administered PO at approximately 30 mg/kg (dose range, 30.0-30.8 mg/kg) via 60 mL catheter tip syringe (Monoject Medtronic, Minneapolis, MN) mixed with water and dark corn syrup (Karo, ACH Food Companies, Inc. Cordova, Tennessee) to a total volume of 60 mL. Flunixin meglumine paste (FLU; Flunazine Equine Paste; Bimeda, Inc., Le Sueur, Minnesota) was administered PO at approximately 1.1 mg/kg (dose range, 1.1-1.19 mg/kg). Placebo (PLAC, Karo Syrup, ACH Food Companies, Inc., Cordova, Tennessee.) was a mixture of water and dark corn syrup administered via catheter tip syringe as described earlier.

2.4 | Sample collection for plasma drug concentrations

Blood samples (approximately 20 mL of waste sample and 8 mL of sample) were collected from the dedicated jugular vein catheter by aspiration from the catheter through the extension set at the following time points: before acetaminophen administration (time 0), and at 15, 30, 60 minutes, and 2, 4, 6, and 8 hours after oral drug administration. Catheters were flushed with 12 mL heparinized saline (10 U/mL heparin in 250 mL 0.9% sodium chloride solution) after each sample collection. The catheter was removed after the final sample collection at 8 hours. A final sample was obtained at 24 hours via percutaneous jugular venipuncture. Blood samples were injected into heparinized tubes (BD Vacutainer Heparin Tubes, BD, Franklin Lakes, New Jersey) within 30 seconds of collection, and stored on ice for a maximum of 2 hours until centrifugation for collection of plasma, then stored at −80°C until analysis. Samples were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) for determination of acetaminophen concentrations.

2.5 | Analysis of plasma concentrations of acetyl-para-aminophenol

Plasma concentrations of acetyl-para-aminophenol (APAP) were measured by liquid chromatography with tandem mass spectrometry (LC MS/MS, Acquity UPLC and Acquity QDa mass spectrometer, Waters corporation, Milford, Massachusetts) as previously described.[16] All the samples were analyzed on the same analytical day. Standard curves were linear over a concentration range of 0.15 to 30 μg/mL for APAP with an $R^2 \geq .999$. The lower limit of quantification (LLOQ) for APAP was 0.15 μg/mL and the lower limit of detection (LLOD) was 0.003 μg/mL. All standard concentrations were calculated to within ±12% of the true concentration. For APAP at concentrations of 0.15, 7.5, and 30 μg/mL, the intraday accuracy was 2.4 ± 1.6%, 2.6 ± 0.67%, and 1.6 ± 1.2%, respectively. For APAP at concentrations of 0.15, 7.5, and 30 μg/mL the intraday precision was 2.4, 0.69, and 1.2, respectively.

2.6 | Pharmacokinetic data analysis

Acetaminophen plasma concentration data was analyzed by noncompartmental analysis with commercially available software (Phoenix WinNonlin 8.2; Pharsight Corporation, Cary, North Carolina). The area under the plasma concentration vs time curve extrapolated to infinity ($AUC_{0-\infty}$) for each dose was calculated by the linear trapezoidal rule. Additional parameters reported include the terminal phase rate constant (λ_z), half-life of the terminal phase ($T_{½}$), and mean residence time. Because an IV dose was not administered, bioavailability (F%) could not be calculated and the volume of distribution and clearance are reported as V_d/F and Cl/F, respectively. The time to maximum plasma concentration (T_{max}) and the maximum plasma concentration (C_{max}) were reported directly from the data. λ_z was reported as arithmetic mean (range), and the remainder of parameters were reported as geometric mean (range). Dose proportionality to a previously published study by acetaminophen at 30 mg/kg PO in healthy horses[16] was calculated by the dose normalized analysis of variance for 2 doses approach from Smith et al for $AUC_{0\text{-}8\ hours}$.[25] A 90% confidence interval was calculated for each variable, and acceptable range of dose proportionality was set at 80% to 125%.[26]

2.7 | Sample collection and analysis for clinicopathologic data

For all treatments, blood samples (approximately 20 mL of waste sample and 4-24 mL of sample) were collected from a dedicated jugular vein catheter for sample collection by aspiration from the catheter through the extension set. Blood was collected in EDTA tubes (BD Vacutainer EDTA Tubes, BD, Franklin Lakes, New Jersey) for a complete blood count at time-120 (the time of LPS model induction), time-105, time-90, time-60, and time-30 minutes, and 0, 1, 2, 4, 6, and 8 hours after treatment administration and run on a commercially available automated analyzer (Biochem Immunosystems Hematology, Montreal, Canada), immediately after collection. Additionally, differentials for the white blood cell lines were performed manually on hematoxylin and eosin-stained slides. Blood was collected into EDTA tubes (BD Vacutainer EDTA Tubes, BD, Franklin Lakes, New Jersey) for serum amyloid A (SAA) measurement at time − 2 (the time of LPS administration), and at 2, 8, and 24 hours after treatment administration and analyzed within 2 minutes after collection per manufacturer's instructions (Stablelab handheld reader, Zoetis Inc, Kalamazoo, MI). Blood was collected into heparinized tubes (BD Vacutainer Heparin Tubes, BD, Franklin Lakes, New Jersey) at time-2, time-1, time-0, time-2, and time-6 hours after treatment administration for determination of blood lactate concentrations

utilizing a hand-held point of care lactate meter (Lactate Plus Vet; Nova Biomedical, Waltham Massachusetts) within 2 minutes of collection.

Blood was collected into siliconized glass tubes (BD Vacutainer Serum Tubes, BD Franklin Lakes, New Jersey) for cytokine multiplex analysis at −2, 0, 4, and 8 hours after treatment administration. Whole blood in siliconized glass tubes was allowed to clot for 30 minutes, and samples were centrifuged at 1000 g for 10 minutes. Serum was removed, and samples were stored in polypropylene cryogenic vials (Fisherbrand Cryogenic Storage Vials, Fisher Scientific, Hampton, New Hampshire) at −30°C until cytokine multiplex analysis with a commercially available equine-specific multiplex cytokine/chemokine magnetic bead kit (MilliporeSigma, Merck KGaA, Burlington, Massachusetts) to simultaneously test for 5 different cytokines in serum samples. All samples were analyzed in triplicate with a 96-well plate format performed per manufacturer's instructions. Sample volume used was 25 µL, and all samples were run undiluted per manufacturer's instructions. Plates were read with a Luminex MagPix instrumentation (Luminex Corporation, Austin, Texas). A minimum bead count of 50 for each cytokine/chemokine was acquired for data analysis. Cytokines included in the panel were IL-1β, IL-6, IL-8, IL-10, and TNF-α. A coefficient of variation (CV) for triplicate samples of <15% were required for data to be included in analysis; when the CV was >15%, samples were either rerun or an outlier sample was removed, and the average of the duplicate sample was used in analysis. Data were analyzed by Milliplex Analyst 5.1 software (Luminex Corporation, Austin, Texas). The lower limit of detection (LLOD) for IL-1 beta was 4.2 pg/mL, for IL-6 was 7.85 pg/mL, for IL-8 was 55.92 pg/mL, for IL-10 was 19.64 pg/mL, and for TNF-α was 3.88 pg/mL. For each timepoint where cytokine values fell below the LLOD, the timepoint was assigned the $\frac{LLOD}{\sqrt{2}}$.[27] If greater than 50% of a treatment's cytokine values were below the LLOD, that cytokine was deemed unsuitable for statistical analysis.

2.8 | Statistical analysis for clinicopathologic samples

As a result of the repeated measures study design, there was a potential for treatment order effects. Therefore, the association between treatment, treatment order, sample time, and clinicopathologic data values were analyzed by the generalized estimating equations (GEE) method to determine the association between treatment, treatment order, and sample time, using commercially available statistical software (SAS, version 8.02 for Windows [PROC GENMOD]; SAS Institute, Cary, North Carolina). The null hypothesis was that there would be no difference in clinicopathologic data values when compared to baseline between treatment groups. Model specifications included a normal distribution, identity link function, repeated statement with subject equal to horse identification, and an exchangeable structure. Treatment was considered a class variable with 3 categories (APAP, FLU, PLAC). Time was also treated as a class variable with 5 categories (1, 2, 4, 6, and 8 hours after treatment). Treatment order was considered a class variable with 3 categories (1, 2, or 3). Differences between treatments at each time point when compared to each treatment's baseline (0 hour) were analyzed by Tukey-Kramer adjustment for multiple comparisons. Significance was set at $P < .05$.

Cytokine data was also analyzed by the GEE method with the software and model specifications described above. The null hypothesis was that there would be no difference in cytokine values between each timepoint between treatment groups. Treatment was considered a class variable with 3 categories (APAP, FLU, PLAC). Time was also treated as a class variable with 3 categories (0, 4, and 8 hours after treatment administration). Treatment order was considered a class variable with 3 categories (1, 2, or 3). Differences between treatments at each time point when compared to each treatment's baseline (0 hour) were analyzed by Tukey-Kramer adjustment for multiple comparisons. Only the variables remaining in the final multivariable model at $P < .05$ are reported.

2.9 | Physical exam data collection and statistical analysis

Horses were continuously monitored for 12 hours after LPS model induction, and every 6 hours until 36 hours after LPS model induction for adverse effects. Complete physical exams were performed every 6 hours for the duration of each 36-hour treatment period. For data analysis, heart rate and rectal temperature were additionally obtained at time-120 (before LPS model induction), time-105, time-90, time-60, and time-30 minutes, and 0, 1, 2, 4, 6, 8, and 24 hours after treatment administration. Heart rate was determined via auscultation over a 15 second period and the number of beats were multiplied by 4 to obtain beats/minute. Rectal temperature was obtained via digital thermometer (VetOne Dual Scale Digital Thermometer; MWI Animal Health, Boise, Idaho).

Heart rate and temperature data were analyzed by the generalized estimating equations (GEE) method to determine the association between treatment, treatment order, and sample time. The null hypothesis tested was that there would be no difference in heart rate or temperature between groups following drug administration at time 0 hour. Data were analyzed by a statistical computer software program (SAS, version 8.02 for Windows [PROC GENMOD]; SAS Institute, Cary, North Carolina). Model specifications included a normal distribution, identity link function, repeated statement with subject equal to horse identification, and an exchangeable structure. Treatment was considered a class variable with 3 categories (APAP, FLU, PLAC). Time was also treated as a class variable with 6 categories (1, 2, 4, 6, 8 and 24 hours after treatment administration) for temperature and heart rate. Trial number was also treated as a class variable with 3 categories corresponding to the order of each treatment. Differences between treatments at each time point when compared to each treatment's baseline (0 hour) were analyzed by Tukey-Kramer adjustment for multiple comparisons. Significance was set at $P < .05$.

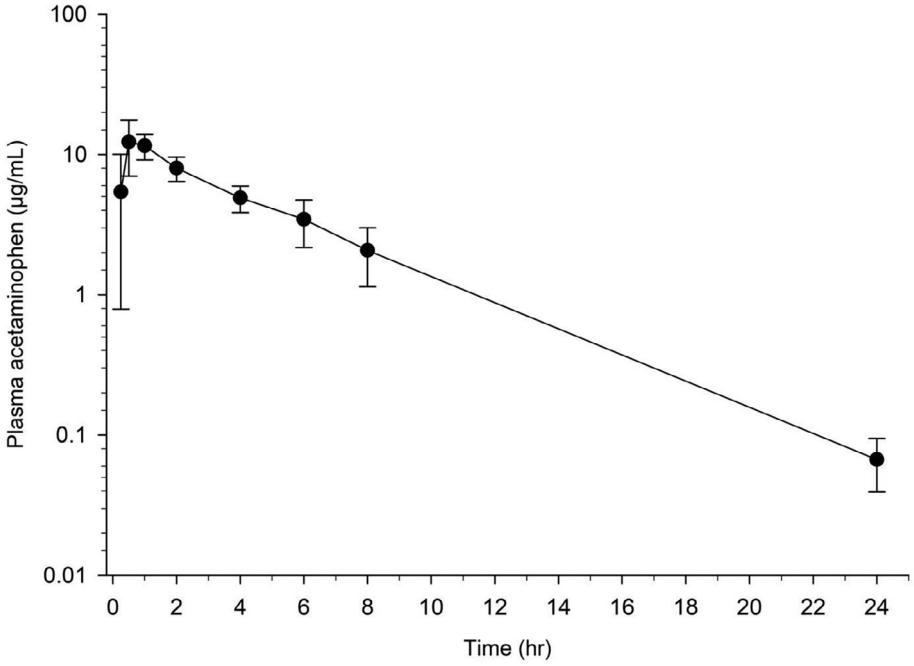

FIGURE 1 Plasma concentration vs time curve for single dose acetaminophen (30 mg/kg per os) for n = 8 horses with experimentally induced endotoxemia, presented as mean ± SD

3 | RESULTS

3.1 | Acetaminophen pharmacokinetics

Plasma APAP concentrations reached a peak geometric mean concentration of 13.97 µg/mL (range, 11.60-20.69 µg/mL) within a geometric mean of 0.65 hour (range, 0.5-1 hour) of administration for a single 30 mg/kg dose (Figure 1). The elimination half-life was a geometric mean of 3.11 hours (range, 2.7-3.5 hours). Additional pertinent pharmacokinetic parameters for APAP are presented in Table 1. The $AUC_{0\text{-}8}$ was not found to be dose proportional between studies[18] with an average ratio of dose-normalized values of 0.36 ± 0.14 and a calculated 90% CI (27.9%-43.5%).

TABLE 1 Noncompartmental pharmacokinetic parameters for acetaminophen (30 mg/kg) given PO as a single dose to adult horses (n = 8) with experimentally induced endotoxemia

Parameter	Geometric or arithmetic mean (range)
T_{max} (hour)	0.65 (0.50-1.00)
C_{max} (µg/mL)	13.97 (11.60-20.69)
λz (hr^{-1})	0.22 (0.19-0.26)
$T_{1/2}$ (hour)	3.11 (2.70-3.50)[a]
AUC_{last} (hr µg/mL)	61.09 (46.39-83.52)
$AUC_{0\text{-}\infty}$ (hr µg/mL)	61.38 (46.5-83.92)
V_d/F (L/kg)	2.19 (1.62-2.69)
Cl/F (mL/kg/min)	8.14 (5.96-10.05)
MRT (hour)	4.31 (3.53-5.16)

Note: λ_z is reported as arithmetic mean (range), the remainder of parameters are reported as geometric mean (range).
Abbreviations: $AUC_{0\text{-}\infty}$, area under the concentration-time curve extrapolated to infinity; AUC_{last}, area under the curve until last measured timepoint; Cl/F, clearance dependent on bioavailability; C_{max}, maximum concentration; MRT, mean residence time; $T_{½}$ λz, half-life of terminal phase; T_{max}, time to maximum concentration; V_d/F, apparent volume of distribution dependent on bioavailability; λz, slope of the terminal phase.
[a]Harmonic mean = 3.09 hours.

3.2 | Physical exam

There was a significant treatment order effect for rectal temperatures, which was accounted for in the GEE model. There were no significant differences in rectal temperature between all treatments (APAP, FLU, and PLAC) from time 0 (before LPS administration) to up to 2 hours after treatment administration (Table S1). At 4 and 6 hours after treatment, both APAP (P = <.001, P = .03, respectively) and FLU (P = .0045 and P < .001, respectively) had a significantly greater decrease in rectal temperature than PLAC when treatments were compared to the time of drug administration (Figure 2). There were no significant differences between treatments at time 10 and 24 hours.

There was a significant treatment order effect for heart rate, which was accounted for in the GEE model. There were no significant differences in heart rate between treatments from the time of LPS administration (0 hour) to the time of drug administration (2 hours). At 4 hours (P = .004) and 6 hours (P = .035) after treatment, FLU had a greater decline in heart rate when compared to APAP (Figure 3). At 4 hours after treatment FLU had a greater decline in heart rate when compared to PLAC (P = .047; Figure 3). There were no other statistically significant differences detected. Average heart rate data is reported in Table S2.

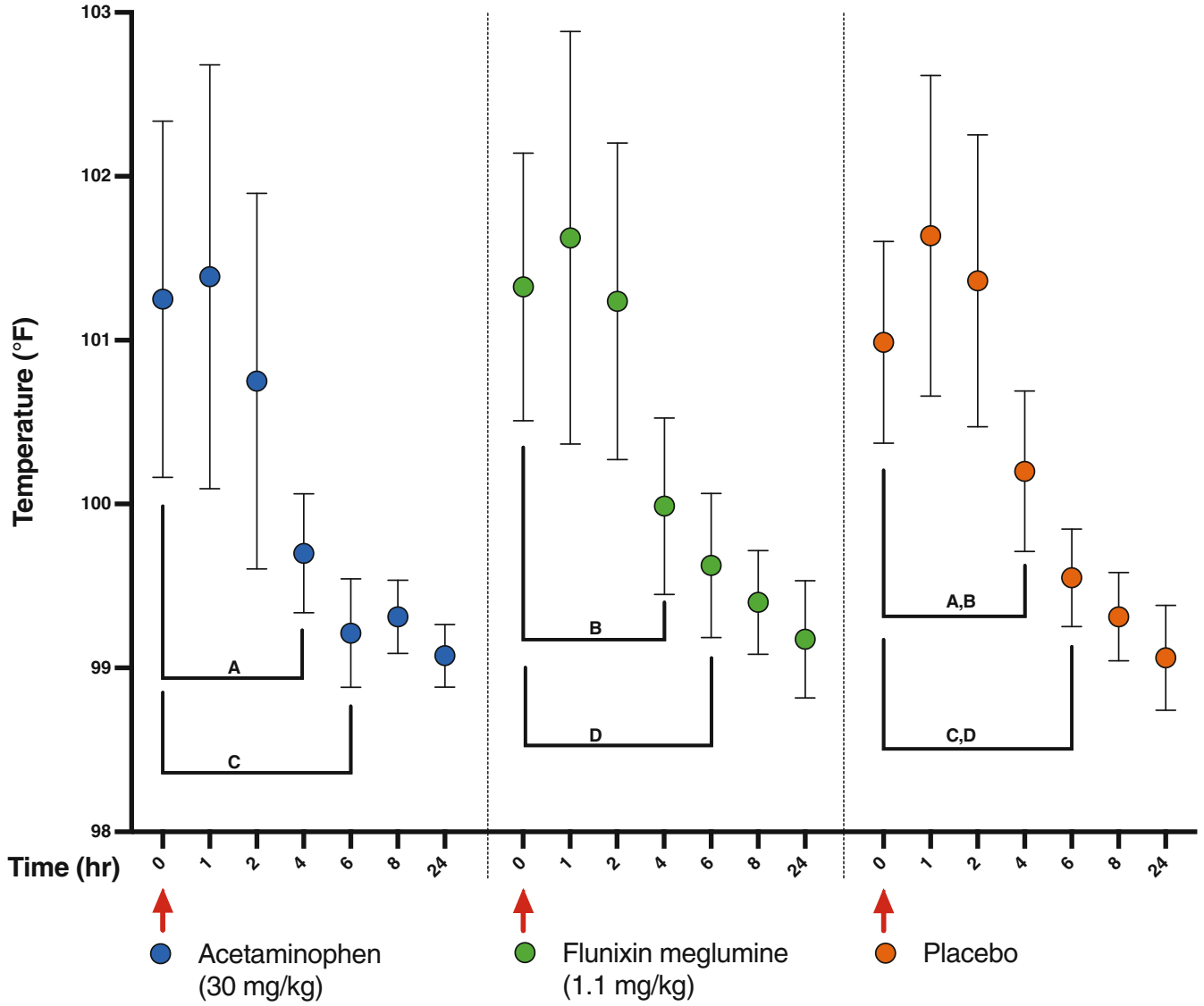

FIGURE 2 Rectal temperature from time of treatment administration (arrow) for n = 8 horses with experimentally induced endotoxemia, presented as mean ± SD. [A-D]Significant change in rectal temperature from the time of drug administration for pairwise comparisons between treatments; $P < .05$

3.3 | Hematology and serum biochemistry

When compared to the time of drug administration, there were no statistically significant differences between treatments at any timepoint for white blood cell (WBC), band neutrophils, total protein, fibrinogen, SAA, or lactate. Values for selected laboratory data are available in Table S3.

For all between treatment comparisons, the difference in analyte value at each timepoint from the time of drug administration was evaluated. At 4 hours after treatment, the neutrophil count was significantly lower for APAP than PLAC ($P = .03$). The lymphocyte count was significantly lower for APAP than FLU ($P = .008$), and significantly lower for APAP than PLAC at 1, 2, and 4 hours after treatment ($P = .003$, $P = .01$, and $P = <0.001$, respectively). The monocyte count remained within normal reference range for all treatments. The monocyte count was significantly lower for APAP than PLAC ($P = .03$) at 2 hours after treatment, and significantly lower for FLU than PLAC ($P = <.001$) at 8 hours after treatment. The eosinophil count remained within normal reference range for all treatments. The eosinophil count was significantly lower for APAP than FLU ($P = .009$) at 2 hours and significantly lower for APAP than PLAC ($P = .01$) at 6 hours after treatment.

The total red blood cell (RBC) count remained within normal reference range for all treatments. The RBC count was significantly lower for APAP than FLU ($P = .004$) and significantly lower for APAP than PLAC ($P < .001$) at 1 hour after treatment, and significantly higher for APAP than PLAC ($P < .001$) at 2 hours after treatment. The hemoglobin (Hgb) concentration remained within normal reference range for all treatments. The HgB concentration was significantly lower for APAP than FLU ($P = .03$) and significantly higher for APAP than PLAC

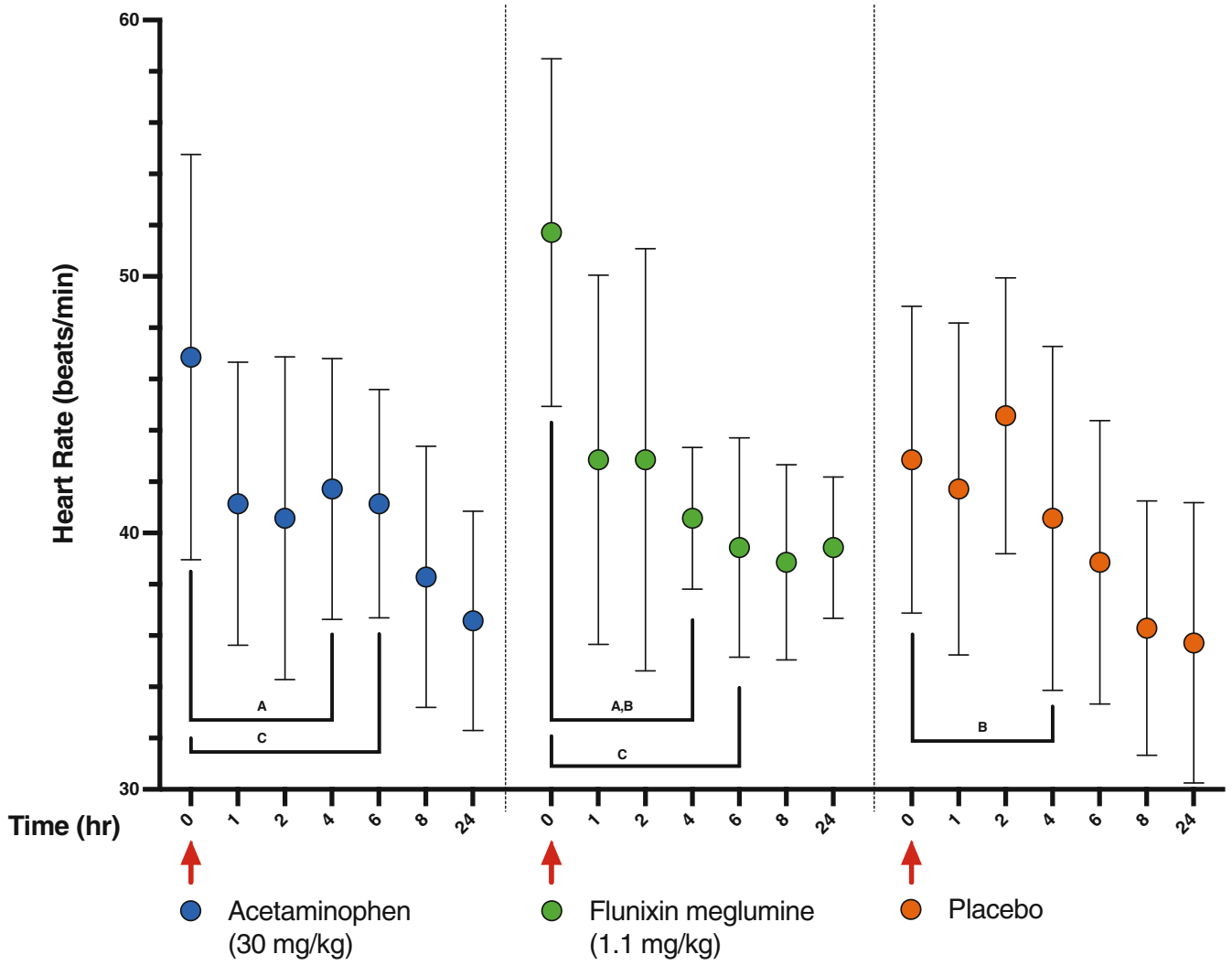

FIGURE 3 Heart rate from time of treatment administration (arrow) for n = 8 horses with experimentally induced endotoxemia, presented as mean ± SD. [A-C]Significant change in heart rate from the time of drug administration for pairwise comparisons between treatments; $P < .05$

at 1 and 2 hours after treatment ($P = <.001$ and $P = <.001$, respectively). Hematocrit remained within normal reference range for all treatments. Hematocrit was significantly lower for APAP than FLU ($P = .003$) and significantly higher for APAP than PLAC at 1, 2, and 6 hours after treatment ($P = <.001$, $P = <.001$ and $P = .04$, respectively). The platelet count remained within normal reference range for all treatments. Platelet count was significantly higher for APAP than PLAC ($P = .03$) and significantly higher for FLU than PLAC ($P = .02$) at 2 hours after treatment. Only IL-1β, IL-10, and TNF-α were suitable for statistical analysis. There were no significant differences between any treatments at any time point for IL-1β, IL-10, and TNF-α. Cytokine values for all horses are reported in Table S4.

4 | DISCUSSION

Acetaminophen was rapidly absorbed after oral administration to horses with experimentally induced endotoxemia. However, the maximum plasma concentration achieved after a single oral dose of 30 mg/kg in this study (geometric mean 13.97 μg/mL [range, 11.60-20.69 μg/mL]) is lower than that achieved in a previous study of healthy horses with a mechanically induced lameness (geometric mean 30.02 μg/mL [range, 14.78-60.01 μg/mL]).[16] The time to maximum plasma concentration (T_{max}) was similar in this study (geometric mean 0.65 hour [range, 0.5-1 hour]) compared to the previous study (0.43 hour [range, 0.25-1 hour]).[16] Because of the different sampling windows between this study (24 hours) and the previous study (8 hours), no direct comparison could be made for the area under the $AUC_{0-\infty}$.[16] When calculating the AUC for the first 8 hours of sampling for this study, the geometric mean $AUC_{0-8\ hours}$ was found to be 44.8 hr μg/mL (range, 38.0-60.3 hr μg/mL), which is substantially lower than previously reported for a 30 mg/kg dose of acetaminophen in horses with mechanically induced lameness [geometric mean 128.04 hr μg/mL (range, 71.9-176.2 hr μg/mL)].[16] The lack of dose proportionality between this study and a previous study in healthy horses suggests that systemic

administration of endotoxin might alter acetaminophen pharmacokinetics in horses.[16]

The effect of endotoxin administration on acetaminophen absorption kinetics has been reported in lambs[27] and humans[28] where there was a significant increase in T_{max} and decrease in C_{max} and AUC for endotoxemic individuals when compared to healthy individuals. These studies suggested that the decreased absorption of acetaminophen in endotoxemic individuals could be because of a decrease in abomasal or gastric emptying rate, the rate limiting step in acetaminophen absorption. Given the similarity in the T_{max} between the previous study in healthy horses and this study in endotoxemic horses, it is unlikely that alteration in gastric emptying rate was a major underlying cause of the reduction in C_{max} and AUC observed. It is more likely that the reduced gastric and small intestinal perfusion in endotoxemic animals, coupled with venous congestion, leads to decreased absorption of enterally administered drugs in endotoxemia.[29,30]

This study was designed to mimic a clinical drug administration scenario, where all treatments were given 2 hours after LPS infusion at the height of the pyretic response. The advantage of this study design rather than a pretreatment design was that it allowed for evaluation of the efficacy of these treatments at alleviating the clinical signs of endotoxemia, rather than evaluating their ability to mitigate the development of these clinical signs. The oral route of administration meant that there would be a lag in maximum drug effect—which might have differed between drugs and horses and could result in a possible normalization of the LPS effect. Despite the establishment of endotoxemia before treatment administration and these concerns of a lag in maximal effect, acetaminophen at 30 mg/kg PO caused a significant decrease in rectal temperature at 4 and 6 hours after treatment when compared to placebo and was not found to be statistically different to flunixin meglumine as an antipyretic in horses with endotoxin-induced pyrexia. In children, the maximum antipyretic response lags behind the maximum plasma concentration by 2.5 hours for both acetaminophen and ibuprofen.[15] Whereas a rectal temperature reading was not taken at 3 hours after treatment in this study, there is a similar timing of the maximum antipyretic effect (4 hours) in horses for acetaminophen and flunixin meglumine. In humans with endotoxemia, acetaminophen combined with the nonselective COX inhibitor ibuprofen provides faster and more effective antipyresis than either monotherapy with a low incidence of adverse effects.[31]

Treatment with flunixin meglumine resulted in a greater reduction in heart rate than acetaminophen at 4 and 6 hours after treatment and a greater reduction in heart rate than placebo at 4 hours after treatment. IV administration of flunixin meglumine effectively decreased endotoxin-induced tachycardia in horses when compared to the phosphodiesterase inhibitor pentoxifylline.[32] Whereas acetaminophen IV decreases heart rate in critically ill humans when compared to placebo, there have not been any prospective studies comparing differences in heart rate reduction between acetaminophen and nonselective COX inhibitors in any species.[33] Because there were no significant differences detected in reduction in rectal temperature between acetaminophen and flunixin meglumine in this study, it is likely that the greater reduction in heart rate for flunixin meglumine is because of its greater systemic COX inhibitory effects than acetaminophen.[34] Specifically, thromboxane A_2 (TXA_2) has a direct positive chronotropic effect in LPS-treated mice, indicating that prostanoids have a direct effect on cardiac pacemaker cells.[35] In horses treated with flunixin meglumine IV before endotoxin administration, there was a significant suppression of TXB_2 (the stable metabolite of TXA_2) when compared to placebo treated horses.[34] Therefore, it is assumed that flunixin meglumine would have a greater effect on circulating TXA_2 than acetaminophen, accounting for flunixin meglumine's greater effect on heart rate.

There was no significant difference in change in white blood cell count after treatment administration for any of the treatments at any time point. The effect of flunixin meglumine on total leukocyte count is not significantly different from placebo in horses following a low dose endotoxin challenge.[36] In this study, neutrophil counts were within the reference range for all treatments by 1 hour after treatment administration. The significantly greater increase in neutrophil count from time 0 for placebo when compared to acetaminophen at 4 hours after treatment administration represents a more profound postneutropenia rebound for placebo than acetaminophen. This is further demonstrated by average neutrophil counts being above the reference range for placebo at 6 hours after treatment, whereas neutrophil counts of horses treated with acetaminophen and flunixin meglumine did not exceed the reference range at any time point.

Acetaminophen treatment resulted in a greater degree of lymphopenia than flunixin meglumine and placebo at 1 hour after treatment, and greater degree of lymphopenia than placebo at 2 and 4 hours after treatment. Acetaminophen damages lymphocyte DNA in vitro[37] and in vivo[38] in humans after hepatotoxic doses but is protective against lymphocyte DNA damage in trauma patients at therapeutic dosing.[39] The pathophysiology and clinical relevance of the lymphocyte changes in this study in horses is unknown.

The clinical relevance of the greater increase from time 0 in average monocyte count for placebo in comparison to acetaminophen at 2 hours after treatment is unknown, however average monocyte counts did not range outside of the reference interval for any treatment at any time point. Similarly, while there were statistically significant differences in eosinophil counts between acetaminophen and flunixin meglumine at 2 hours after treatment, and for acetaminophen and placebo at 6 hours after treatment, average eosinophil counts did not range outside of reference range at any time point. There was a significantly greater increase in hematocrit and RBC count for both flunixin meglumine and placebo when compared to acetaminophen at 1 hour after treatment. Additionally, there was a significantly greater increase in hematocrit and RBC count for placebo in comparison to acetaminophen at 2 hours after treatment, and a greater increase in hematocrit for placebo compared to acetaminophen at 6 hours after treatment. Whereas none of these values varied outside of reference range, the evidence of greater hemoconcentration for flunixin meglumine and placebo could have been because of altered water consumption behavior, alterations in hemodynamics, or fluid shifts. The greater increase in platelet count for acetaminophen and flunixin meglumine in comparison to placebo 2 hours after treatment is of

unknown clinical relevance, however average platelet count remained within the reference range for all time points examined.

Whereas there were no significant differences in SAA concentrations between treatment groups, SAA concentrations rose substantially to greater than 150 μg/mL 10 hours after LPS exposure, and >500 μg/mL 24 hours after LPS exposure, for all treatments. The timing of this increase is similar to other reports, where SAA concentrations rose significantly above baseline at 6 hours after LPS exposure and were >1000 μg/mL at 24 hours after LPS exposure.[40] This finding is indicative of the activation of the acute phase response under a transient sterile inflammatory state and underscores the importance of accurate interpretation of SAA in horses.

There were no significant differences between treatments for the cytokines suitable for statistical analysis (IL-1β, IL-10, and TNF-α) in this study. Flunixin meglumine prevents the increase in IL-10, IL-1β, and TNF-α in mice with experimentally induced endotoxemia,[41] however, this effect has not been replicated in vivo.[42] Therefore, whereas flunixin meglumine and acetaminophen were found to reduce the rectal temperature in this model of endotoxemia, neither treatment prevented the endotoxin-mediated increases in proinflammatory cytokines in this study.

The major limitations of this study include the small sample size, and the Latin square study design. The crossover design was advantageous in that it eliminated inter-horse variability and allowed for each horse to be used as its own internal control. However, there was still evidence of endotoxin acclimation despite the 30-day washout period between treatments as evidenced by strong treatment order effects requiring correction in the statistical analysis. The lack of IV dosing of acetaminophen and the lack of pharmacokinetic data from these horses without endotoxin administration is a limitation in understanding the pharmacokinetics of this drug in endotoxemic horses. This study also did not include the FDA labeled treatment for pyrexia in horses, dipyrone. Whereas acetaminophen and dipyrone are thought to have a similar mechanism of action, dipyrone was not included in this study because of a lack of an oral dose formulation.

Acetaminophen at 30 mg/kg PO was found to be superior to placebo and not statistically different from flunixin meglumine (1.1 mg/kg PO) in reducing rectal temperature in adult horses with experimentally induced endotoxemia and could be an option for antipyresis in clinical cases, particularly when administration of traditional NSAIDs is contraindicated. Flunixin meglumine was superior to acetaminophen at reducing endotoxin-mediated tachycardia at 4 and 6 hours after treatment. Acetaminophen administered at 30 mg/kg PO to adult horses with experimentally induced endotoxemia is an effective antipyretic but is unlikely to provide any alteration in systemic inflammatory response. Further research is needed to determine the safety and efficacy of combined or alternating treatment with acetaminophen and COX inhibitors in horses.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the IACUC of Virginia Polytechnic Institute and State University.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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