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Oral administration of tepoxalin in the horse: A PK/PD study

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ABSTRACT

Tepoxalin is a non-steroidal anti-inflammatory drug with analgesic, anti-inflammatory, and antipyretic properties and has been recently introduced into veterinary medicine. The aim of this study was to evaluate the pharmacokinetic/pharmacodynamic (PK/PD) profile of tepoxalin to assess whether it would be suitable for clinical use in horses. Six female fasting/fed horses were given 10 mg/kg tepoxalin orally in a cross-over study. After administration, tepoxalin underwent rapid and extensive hydrolytic conversion to its carboxylic acid metabolite RWJ-20142. In animals that had been fed, the plasma concentrations of tepoxalin were undetectable, whereas in fasting animals they were close to the limit of quantification of the method. No differences between the fasting/fed groups in RWJ-20142 plasma concentrations were shown. Tepoxalin showed a strong and long-lasting ex vivo inhibitory activity against cyclooxygenase (COX)-1, mainly due to its main metabolite RWJ-20142. Tepoxalin and RWJ-20142 do not seem to possess either COX-2 or 5-lipoxygenase inhibitory activity in the horse. These features suggest that the drug is a selective COX-1 inhibitor in horses, with no significant anti-inflammatory activity. Thus, its long term use in equine practice could be of concern.

Keywords: Tepoxalin, RWJ-20142, Horse, Pharmacokinetics, Pharmacodynamics, PK/PD

INTRODUCTION

Chronic inflammatory diseases, such as osteoarthritis, are among the most common causes of reduced function in animals, especially horses (Richardson and Loinaz, 2007). Inflammation, pain, joint alteration/deformation, and impaired locomotion with variable (but usually worsening) degrees of incapacitation are common symptoms. The main aim of current therapeutic strategies is to alleviate the symptoms, particularly those of pain and inflammation. The most commonly used remedies are non-steroidal anti-inflammatory

drugs (NSAIDs) (Goodrich and Nixon, 2006). However, NSAIDs can cause serious adverse effects, the most important of which are gastric lesions (e.g. ulceration), renal failure, and asthma (Lascelles et al., 2005). Both the adverse and therapeutic effects of NSAIDs are primarily the result of the inhibition of the activity of prostaglandin (PG) endoperoxide synthase, or cyclooxygenase (COX) (Brooks and Day, 1991). Inhibition of the constitutively expressed COX-1 isoform causes gastric and intestinal mucosal damage and renal toxicity,

whereas inhibition of the inflammatory-induced COX-2 isoform reduces inflammation. Arachidonic acid not metabolised to prostaglandins by the COX enzymes may enter into the lipoxygenase (LO) pathway, increasing the formation of leukotrienes (LTs) and cysteinyl-LT (Rainsford, 1987, 1993). This causes vasoconstriction at the level of gastric mucosa and increases the formation of reactive oxygen radicals from the peroxidative cleavage of hydroxyeicosatetraenoic acids (Los et al., 1995), resulting in further mucosal injury. The gastric and intestinal mucosal lesions produced by NSAIDs can be prevented by the concurrent administration of 5-LO inhibitors (Rainsford, 1987, 1993). 5-LO converts free arachidonic acid to the unstable intermediate 5-S-hydroperoxyeicosatetraenoic acid (5-HPETE), that is subsequently converted to LTA₄, the 5,6 epoxide of arachidonic acid, which is also unstable (Goldyne, 1984; Stenson and Parker, 1984; Higgs and Moncada, 1985). Depending on the constituent enzymes, LTA₄ is then converted to a variety of LT derivatives, including LTB₄ a potent chemotactic agent associated with inflammation that recruits, activates, and prolongs the actions of neutrophils and other inflammatory cells up-regulating the cytokine production (Crooks and Stockley, 1998; Bertolini et al., 2001). Tepoxalin, 5(4-chlorophenyl)-N-hydroxy-(4-methoxyphenyl)-N-methyl-1H-pyrazole-3-propanamide (Fig. 1), is a NSAID with analgesic, anti-inflammatory, and antipyretic properties that has recently been marketed for use in veterinary medicine. Tepoxalin has been approved for use in dogs by the European Committee for Medicinal Products for Veterinary Use (Zubrin, Schering-Plough) (Zubrin, EPARs/02/10/08). Due to its ability to inhibit both COX isoforms and 5-LO, it is considered a dual inhibitor (Wallace et al., 1993). By reducing the production of LTB₄, tepoxalin might offer a novel approach to both the preservation of GI mucosal integrity and reduction of bronchoconstriction (Wallace et al., 1993; Bertolini et al., 2001; Lees, 2009). Tepoxalin is supplied as a rapidly disintegrating tablet that completely dissolves within a few seconds in the animal's mouth (Anonymous, 2003). Studies conducted in rats (Knight et al., 1996), chickens (De Boever et al., 2009), rabbits (Pollock et al., 2008), dogs (Knight et al., 1996; Homer et al., 2005) and humans

(Waldman et al., 1996) reported that the parent compound reached maximum plasma concentrations in a variable time (0.7–4 h), and was rapidly converted to the active metabolite RWJ-20142. The dual activity of tepoxalin and the potent COX-1 activity inhibition of its metabolite have been demonstrated in different studies (Argentieri et al., 1994; Willburger et al., 1998; Goodman et al., 2009), and the long plasma half-life of the active metabolite allows once-a-day dosing (Homer et al., 2005). According to Waldman et al. (1996), only tepoxalin (and not RWJ-20142) contributes to 5-LO inhibition. However, Willburger et al. (1998) demonstrated that in human blood, although a low plasma concentration of tepoxalin can inhibit COX enzymes, the same concentration did not affect 5-LO inhibition. Agnello et al. (2005) showed that in dogs administration of 10 mg/kg of tepoxalin once daily for 10 days significantly reduced PGE2 production but not LTB4. In the dog, tepoxalin has been shown to be effective and safe in its therapeutic range (Goodman et al., 2009) after single (Homer et al., 2005) and repeated (Knight et al., 1996) administrations. Tepoxalin has been experimentally used in chickens at high dosages (30 mg/kg) with no adverse effects reported (De Boever et al., 2009). Several differences have been reported in pharmacokinetic (PK) parameters between species (Waldman et al., 1996; Homer et al., 2005; Pollock et al., 2008; De Boever et al., 2009), so species-specific data are required. In the horse, there has been just one published study (Caruso et al., 2009). This was a brief in vitro study which reported only the pharmacodynamic properties (PD) of tepoxalin, while data concerning the dynamics of RWJ-20142 were missing. In vitro analyses of PD need to be paired with PK data to predict the clinical response, as the latter influences the concentration of the active molecule within tissues. In fact COX selectivity generally decreases when in vivo assays are compared to in vitro results (Bertolini et al., 2001). This study was undertaken to assess whether tepoxalin has suitable clinical features in horses, by the evaluation of its PK/PD profile using an equine whole blood assay.

MATERIALS AND METHODS

The study protocol was approved by the Ethics Committee of the University of Pisa (authorization 9403) and transmitted to the Italian Ministry of Health.

Animals and experimental design

Six female horses, aged 7–12 years and weighing 450–550 kg, were used. The mares were considered clinically healthy on the basis of a physical examination and complete

haematological analyses. Animals were randomly assigned to two treatment groups, using an open, single-dose, two treatment, two-period, cross-over design. Each subject received a single dose of 10 mg/kg tepoxalin using Zubrin 200 mg tablets (Schering-Plough), bought in an ordinary pharmacy. The first group (n = 3) received the treatment in the morning, after fasting for 12 h overnight up to 6 h after drug administration. The second group (n = 3) was treated in the morning 2 h after feeding. After a wash-out period of 2 weeks the groups were reversed and the treatments repeated. Since no information was available for horses a long wash-out period was chosen. This not only guaranteed the absence of residual plasma concentrations of the drug and its metabolite, but also the lack of residual drug effect on clearance (induction/inhibition) (Toutain and Bousquet-Mélou, 2004). The animals that were fed were provided with a diet composed of a mixture of ground cereals (Equifioc, Molitoria Val di Serchio) in the afternoon (1.5 kg/day) and alfalfa hay in the morning (8 kg/day). Fasting horses were given hay only 6 h after drug administration. All of the animals were given the oral lyophilised formulation dissolved in 100 mL of distilled water via nasogastric tube. After administration, the nasogastric tube was rinsed with 1 L of distilled water to ensure complete delivery of the drug into the stomach. Objective symptoms such as sweating, excitement, trembling, and head nodding were scored using numerical rating scales (Derksen et al., 1999). Vital signs including blood pressure, heart rate, respiratory rate and body temperature were assessed during the study. A catheter was placed into the right jugular vein to facilitate blood sampling. Blood samples for PK analysis (10 mL) were collected at intervals of 0, 5, 15, 30, and 45 min, and 1, 1.5, 2, 4, 6, 8, 10, 24, 34, 48, 58 and 72 h after tepoxalin administration, and placed in collection tubes containing lithium heparin. The blood samples were centrifuged at 400 g for 5 min within 30 min of collection, and the harvested plasma was stored at -20 °C and was used within 14 days from collection. Blood samples for PD analysis were collected at 0, 2, 10, 24, 48 and 72 h after drug administration (n = 3). Blood for the COX-1, COX-2 and 5-LO activities measurement was collected into siliconised glass, sodium-heparinised and lithium-heparinised tubes (Venoject, Terumo), respectively.

Preparation of samples for PD analysis

Aliquots of non-heparinised blood (10 mL) were incubated at 37 °C for 1 h to allow thromboxane (TX)_{B2} production from platelets. Tubes were centrifuged at 400 g for 10 min at room temperature and the serum was stored at -20 °C. Aliquots (2 mL) of sodium-

heparinised blood were transferred into glass tubes containing a final concentration of 10 µg/mL lipopolysaccharide (LPS) from *Escherichia coli* (serotype 0111:B4) (Sigma) in phosphate buffered saline (PBS) solution and incubated at 37 °C for 24 h to simulate PGE2 production from mononuclear cells. Blood with PBS only was used as a negative control. At the end of the incubation period, blood was centrifuged at 400 g for 10 min at room temperature to obtain plasma. A 100 µL aliquot of plasma was mixed with 400 µL of HPLC-grade methanol (Carlo Erba) to precipitate proteins. The mixture was centrifuged at 400 g for 10 min at room temperature; the supernatant was collected and stored at -20 °C. Aliquots (0.5 mL) of lithium-heparinised blood were transferred into glass tubes, containing 60 µM calcium ionophore A23187 (Sigma) in DMSO, to stimulate LTB4 production from neutrophils. Blood with DMSO only was used as the negative control. Tubes were incubated at 37 °C for 30 min. Two millilitres of methanol were added to the mixture to precipitate proteins, and the tubes were immediately immersed in ice to stop the incubation. Tubes were centrifuged at 4000 g for 15 min at 4 °C and aliquots of the resulting supernatant were stored at -20 °C until used. TXB2, PGE2 and LTB4 were assayed using a commercial EIA kit (Assay Designs), in samples diluted 15-, 25- and 10-fold, respectively, using the assay buffer supplied with the kit.

Chromatographic assay

High performance liquid chromatography (HPLC) was performed with a PU 980 isocratic pump (Jasco) attached to an AS-1550 autosampler (Jasco) and an 821-FP fluorimetric detector (Jasco). Integration was performed using Borwin chromatographic software (version 1.21). The compounds were separated on a C18 column (250 mm X 4.6 mm i.d., 5 µm particles) (Gemini) with a mobile phase A, consisting of 0.01 M 1-octane-sulfonic acid (Sigma) in 0.01 M acetic acid (Sigma) in HPLCgrade water (Baker), and a mobile phase B consisting of tetrahydrofuran (Carlo Erba), mixed at 50/50 (v/v). The flow rate of the mobile phase was maintained at 1.0 mL/min. Injection volume was 50 µL and the detector wavelengths were set at 290 nm and 440 nm as excitation and emission, respectively (Giorgi et al., 2011). Briefly, the quantification of tepoxalin and RWJ-20142 in plasma samples was accomplished by chromatographic analysis of unknown samples in parallel with standard curve and quality control samples created using tepoxalin, RWJ-20142 and RWJ-20294 (internal standard) pure standard powders (TopChem Laboratories). For each series of analyses, a standard curve was generated as well as

nine quality control samples (three different concentrations) alongside the test samples. The limits of detection (LOD) and quantification (LOQ) were determined as analyte concentrations giving signal-to-noise ratios of 3 and 10, respectively.

Preparation of samples for PK analysis

The drug was extracted from horse plasma by the following procedure. The plasma sample (0.5 mL) was spiked with 10 µg/mL RWJ-20294 as an internal standard (100 µL) in a 2 mL plastic Eppendorf tube. The mixture was vortexed for 30 s and left to rest for 5 min. Acetonitrile (0.9 mL) (Carlo Erba) was added to the mixture as an extraction solvent. This mixture was vortexed, shaken for 10 min at 150 oscillation/min on a shaker, and then centrifuged at 5000 g at 4 °C for 15 min. Four hundred microlitres of the upper layer were transferred to a clean Eppendorf and centrifuged again for 15 min at 5000 g at 4 °C. Two hundred microlitres were separated in a 500 µL Eppendorf. The aqueous phase (50 µL) was then injected onto the HPLC system.

PK evaluation

The pharmacokinetic calculations were carried out using WinNonLin v 5.2.1 (Pharsight Corp). Maximum concentration (C_{max}) of tepoxalin and RWJ-20142 in plasma, and the time required to reach C_{max} (T_{max}) were predicted from the data. The terminal rate constant (λ) was determined from the slope of the terminal phase of the plasma concentration curve that included a minimum of three points. The half-life of the terminal phase (T_{1/2λ}) was calculated by the formula $T_{1/2} = 0.693/\lambda$. The area under the concentration/time curve (AUC_{last}) was calculated using the linear trapezoidal rule. Changes in plasma concentrations of tepoxalin and RWJ-20142 were evaluated using the standard non-compartmental analysis, and the relative pharmacokinetic parameters were determined using standard non-compartmental equations (Gabrielsson and Weiner, 2002).

Statistical analysis

Normal distribution of data was assessed by the D'Agostino and Pearson omnibus normality test. Consequently, the differences between the pharmacokinetics of tepoxalin during the two periods of administration, as well as the effect on prostanoid levels and

percentage inhibition of prostanoid production were calculated using a repeat measures ANOVA, followed by the Newman–Keuls multiple comparison test. The effect of fasting/feeding status on the results was assessed using the twoway ANOVA with the Bonferroni correction as a post hoc test. A cut-off value of 20% inhibition of COX-1 was taken as the percentage value above which there may be a risk of adverse effects, and a cut-off value of 80% inhibition of both COX-2 and 5-LO was taken as the percentage value above which a good therapeutic effect would be expected (Giraudel et al., 2005). The results are reported as mean values (\pm SEM). All the analyses were conducted using GraphPad InStat v.4.0 (GraphPad Software). In all the experiments, differences were considered significant if the associated P value was <0.05 .

RESULTS

Throughout the study no abnormality ascribable to the drug under study was observed as assessed by the measurement of objective symptoms, vital signs, physical examination and laboratory analysis.

PK analysis

The analytical method had a LOQ of tepoxalin and RWJ-20142 of 25 ng/mL and 20 ng/mL, respectively. The maximum value of the CV in intra-/inter-day assay precision for tepoxalin and metabolite was 5.5%. The plasma concentration vs. time curves for tepoxalin and RWJ-20142 were best fitted to a non-compartmental model. In fasting animals, the plasma profile of tepoxalin showed lower concentrations (Fig. 2). The C_{max} (96.8 ± 55.0 ng/mL) was reached in a short time (T_{max} 1.5 ± 0.2 h) and the drug rapidly disappeared from the blood compartment ($T_{1/2}$ λz 2.78 ± 0.99 h) (Table 1). One of the six horses had all plasma concentrations below the LOQ, and another had only two collection points that were above this level. A wide variation in plasma concentrations among the subjects was observed. The metabolite RWJ-20142 showed a higher C_{max} (1250 ± 187.4 ng/mL), a longer T_{max} (9.6 ± 0.4 h) and a longer $T_{1/2}$ λz (6.3 ± 0.1 h), than the parental compound. In the plasma of fed animals, tepoxalin concentration did not exceed the LOQ of 25 ng/mL in any horse at any time point. In this experimental group, the $T_{1/2}$ λz of RWJ-20142 was significantly ($P = 0.03$) higher (8.8 ± 1.0 h) than in fasting animals (6.3 ± 0.1 h), but the pharmacokinetic profile of RWJ-20142 (Fig. 2) and the other parameters were not

significantly different between the two groups (Table 1). In both treatments, RWJ-20142 showed a biphasic mode of formation: a first rapid phase (1.5–2 h), and a second slower phase culminating with the T_{max} (7.7 h fed group; 9.6 h fasting group). The single curves calculated for each animal showed the same trend (data not shown). The end of the first phase coincided with the T_{max} of the parental drug in fasting horses.

PD analysis

In fasting and fed horses, induced TXB₂ concentrations were 256.3 ± 117.5 ng/mL and 83.9 ± 22.1 ng/mL, respectively; background PGE₂ concentrations were 0.4 ± 0.1 ng/mL and 0.7 ± 0.2 ng/mL, respectively; induced PGE₂ concentrations were 0.7 ± 0.1 ng/mL and 2.4 ± 0.4 ng/mL, respectively; background LTB₄ concentrations were 0.1 ± 0.0 ng/mL and 0.1 ± 0.0 ng/mL, respectively; induced LTB₄ concentrations were 3.6 ± 0.3 ng/mL and 3.1 ± 0.6 ng/mL, respectively. Administration of tepoxalin at 10 mg/kg significantly decreased TXB₂ concentrations at all experimental time points in both fasting (0 h vs. 48 h, $P < 0.05$ and 0 h vs. all other times, $P < 0.01$) and fed ($P < 0.001$) horses compared with the basal values. The maximal inhibition of serum TXB₂ occurred at 24 h and was maintained for up to 72 h after administration (Figs. 3a and 4a). At this time point, serum TXB₂ activity was still inhibited by $90.4 \pm 4.3\%$ and $85.8 \pm 1.1\%$ in fasting and fed animals, respectively (Table 2). Although a reduction in concentrations of PGE₂ was found, this parameter did not decrease significantly within 48 h of drug administration ($P > 0.05$). It reached its maximal inhibition at 10 h (Table 2), both in fasting and fed horses. The inhibition time corresponded to the C_{max} of RWJ-20142. PGE₂ levels reverted back to the stimulated basal values within 72 h of tepoxalin administration, (Figs. 3b and 4b). The concentration of LTB₄ was significantly increased by stimulation with calcium ionophore ($P < 0.001$ and $P < 0.01$ in fasting and fed animals, respectively). In both fasting and fed horses, tepoxalin decreased LTB₄ concentrations 2 h after administration, along with the C_{max} of tepoxalin, even if the values were not statistically significant ($P > 0.05$). At all other times, LTB₄ concentrations were similar to the stimulated basal values (Figs. 3c and 4c). Maximum inhibition of COX-1 ($99.7 \pm 0.1\%$ and $99.6 \pm 0.1\%$ in fasting and fed horses, respectively) exceeded the cut-off value of 20%, whereas the maximum percentage inhibition of both COX-2 ($68.9 \pm 26.6\%$ and $65.1 \pm 8.0\%$ in fasting and fed horses, respectively) and 5-LO ($37.5 \pm 2.9\%$ and $43.4 \pm 4.7\%$ in fasting and fed horses, respectively) did not exceed the cut-off value of 80% (Table 2). Fasting/feeding status significantly affected PGE₂

concentration, accounting for 29.43% of the total variance in PGE2 concentration ($P = 0.0002$). In contrast, it only accounted for 2.93% and 0.83% of the total variance of TXB2 and LTB4 values ($P = 0.11$ and $P = 0.38$, respectively) (Fig. 5a–c).

DISCUSSION

Several variations in PK/PD of NSAIDs among animal species have been reported (Lees et al., 2004a,b). Drugs with selective enzyme-inhibiting features in one species may have nonspecific activity in others. A PK/PD study in the target species is therefore essential for predicting the likely effectiveness and safety of the therapy. In this study PD analysis was carried out using whole blood assays, which tested TXB2 production during clotting, PGE2 production during LPS stimulation and LTB4 production during calcium ionophore stimulation, as indices of the expression of platelet COX-1, monocyte COX-2 and neutrophil 5-LO activities, respectively. This method has the advantage of taking into account the clinically relevant target cells and the variable degree of protein binding of NSAIDs (Blain et al., 2002). In previous *in vivo* studies in other species, tepoxalin has been shown to undergo rapid and extensive conversion to its carboxylic acid hydrolysis product (RWJ-20142). In those studies, plasma concentrations of RWJ-20142 were found to be substantially higher and more persistent than those of the parental drug. The PK findings in this study were in line with those observed in the other species, although the low numbers of animals used mean that the robustness of the PK parameters determined for tepoxalin is limited and further studies in more animals are required to confirm our findings. In horses, the RWJ-20142/tepoxalin C_{max} ratio was about 12, similar to rabbits (Pollock et al., 2008). Studies in dogs (Homer et al., 2005) and humans (Depré et al., 1996; Waldman et al., 1996) reported values closer to five suggesting faster metabolism of tepoxalin in these species. When the AUC of RWJ-20142 obtained in this study was normalised to the orally administered dose of tepoxalin (2756 ng h/mg), the values obtained were similar to those of fasting dogs (2747 ng h/mg) (Homer et al., 2005), but higher than in chickens (1305 ng h/mg) (De Boever et al., 2009) and rabbits (1807 ng h/mg) (Pollock et al., 2008). This suggests that, if the same dose of the drug per kg bodyweight (BW) is administered, RWJ-20142 will have longer plasma persistence in horses and dogs than in chickens and rabbits. Induced TXB2 concentrations measured in the present study were significantly higher when compared to values reported by Brideau et al. (2001) (256.3 ± 117.5 ng/mL and 83.9 ± 22.1 ng/mL in fasting and fed horses,

respectively, vs. 26 ± 12 ng/mL). The high concentration of vehicle (DMSO 0.4%) used in the in vitro experimental design performed by Brideau and co-workers may account for this difference. In fact, it is well known that DMSO possesses anti-inflammatory activity, by inhibiting platelet aggregation induced by arachidonic acid in a concentration-dependent manner (Santos et al., 2003). After drug exposure, the production of TXB2 by platelets was significantly suppressed, but the production of PGE2 and LTB4 by LPS- and calcium-stimulated white blood cells, respectively, was not significantly altered. So our results suggest that an oral dose of 10 mg/kg of tepoxalin can inhibit COX-1 activity in the horse, but not COX-2 and 5-LO, consistent with a significantly different COX inhibition ratio than that reported in other species. However, the large inter-individual variation of in PGE2 production, combined with the small number of animals ($n = 3$), may have resulted in a type II error (Warner, 2008). In the present study, inhibition of TXB2 production was detected for 2–72 h after drug administration, with peak inhibition reached between 2 and 48 h. During this period of time, tepoxalin and its metabolite RWJ-20142 achieved maximal plasma levels, suggesting that both tepoxalin and its acid metabolite could be responsible for the COX-1 activity inhibition. The maximal inhibition of PGE2 occurred at 10 h contemporaneous with the C_{max} of RWJ-20142, whereas that of LTB4 occurred at 2 h contemporaneously with the C_{max} of tepoxalin. In the horse, the only previous report of the PD of tepoxalin in vitro (Caruso et al., 2009), confirmed its preferential inhibitory activity of COX-1 ($IC_{50} = 0.0116 \mu M$). In the same study, the inhibition of the COX-2 ($IC_{50} = 0.0752 \mu M$) was higher than that of 5-LO ($IC_{50} = 0.2233 \mu M$), although in neither case was the inhibition significant. This finding of a preferential inhibition of COX-1 is consistent with the results of the present study (Table 2). However, in this study the inhibition of 5-LO tended to be higher than that of COX-2. This difference may be due to the in vivo metabolism of tepoxalin into its active metabolite RWJ-20142, which has a different PD profile from the parental compound. Comparing the maximum percentage of enzyme inhibitions with the cut-off values suggested by Giraudel et al. (2005), our results suggest that tepoxalin orally administered at a dose rate of 10 mg/kg would have weak anti-inflammatory effects with a relatively high potential for adverse effects. Seventy-two hours after tepoxalin administration, COX-1 activity was still strongly inhibited, in both fasting and fed horses ($90.37 \pm 4.35\%$ and $85.81 \pm 1.12\%$, respectively), despite tepoxalin and RWJ-20142 plasma concentrations both being under the LOQ. There was no such inhibition of COX-2 and 5-LO activities. The mechanism of a prolonged COX-1 activity inhibition for tepoxalin is not fully understood, but it could be related to its non-competitive mode of

COX-1 inhibition, to the inhibition of COX-1 peroxidase (Munroe and Lau, 1995) or to possible covalent binding. Further investigation is required. It is unlikely, that tepoxalin acts like aspirin, (another non-competitive NSAID) as extensive dialysis of COX-1 enzyme after the drug administration can reverse the inhibitory effect of tepoxalin but not that of aspirin (Tam et al., 1995). In the dog, the influence of food has been reported to drastically increase both the bioavailability of tepoxalin and the production of RWJ-20142 (Homer et al., 2005). Diets that are rich in fats have been speculated to reduce the effect of the hepatic first-pass metabolism as a result of the preferential transport of the drug associated with lipoprotein into lymphatic vessels (Porter and Charman, 2001). In the horse, feeding a standard diet, which is low in fat, marginally influenced plasma concentrations of RWJ-20142. However the difference in $T_{1/2kz}$ between groups is likely to be of negligible clinical relevance. Our horses were fed a high fibre diet, which may affect tepoxalin pharmacokinetics (Pollock et al., 2008) and could be the cause of the difference in plasma concentration of tepoxalin between the experimental groups. Feeding also seems to have influenced the basal level of PGE₂, with marked differences between fed and fasting horses. These variations are consistent with early studies highlighting the increase of PGE₂ after corn supplementation (Cargile et al., 2004; Hall et al., 2004). In horses the peak plasma level of tepoxalin was significantly lower than in dogs (0.53–1.08 lg/mL; Homer et al., 2005) and about half that in rabbits (0.21 µg/mL; Pollock et al., 2008), while the $C_{max}(s)$ of RWJ-20142 were consistent among all these species. Two different rate of metabolite formation were shown in both fed and fasted horses. The change in the slope of RWJ-20142 plasma concentration vs. time curves coincided with the beginning of the elimination phase of the parental drug. As soon as the parental drug concentration began to decrease, RWJ-20142 formation began to slowly increase. This could be due to rapid metabolism of tepoxalin in the gastrointestinal tract with subsequent absorption of the active metabolite and/or a higher rate of conversion of tepoxalin to RWJ-20142 than the rate of elimination, distribution and biotransformation of RWJ-20142. In the current study, as in chickens (De Boever et al., 2009), the presence of a time-dependent unknown peak was found in the chromatographic curves of plasma. It eluted between tepoxalin and the acid metabolite, in both fed and fasted horses. The unknown analyte represented approximately 10–15% in the peak area of the active metabolite and its maximal area was simultaneous with the T_{max} of RWJ-20142 (10 h). This peak could potentially represent another active metabolite of tepoxalin. Further studies are needed to identify its molecular structure to further clarify the clinical effectiveness and safety of tepoxalin therapy.

Conclusion In horses dosed orally with 10 mg /kg tepoxalin, the compound is present at very low levels in plasma because it is rapidly converted to its acid metabolite. At the tested dose rate, tepoxalin has both strong and long-lasting ex vivo inhibitory activity against COX-1. These effects seem mainly due to its main metabolite RWJ-20142. Tepoxalin and RWJ-20142 do not seem to possess either COX-2 or 5-LO inhibitory activity suggesting that, at a dose rate of 10 mg/kg BW, tepoxalin would have limited beneficial anti-inflammatory properties in horses, and that its long term use could be matter of concern.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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FIGURES AND TABLES

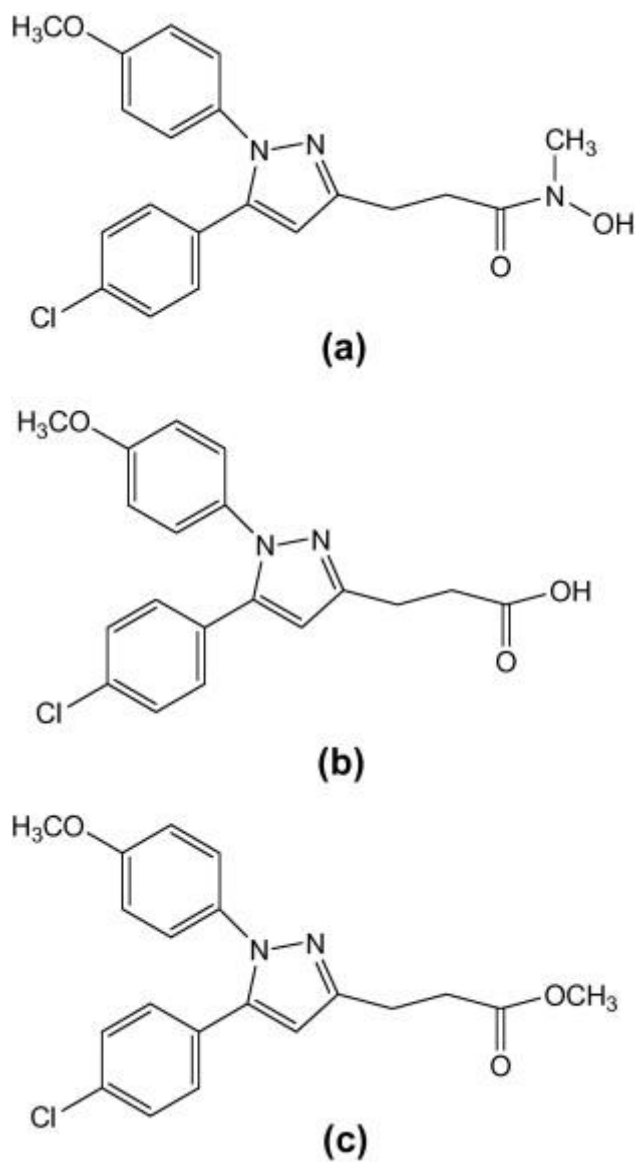


Fig. 1. Molecular structure of (a) tepoxalin, (b) its active acid metabolite (RWJ-20142) and (c) internal standard (RWJ-20294).

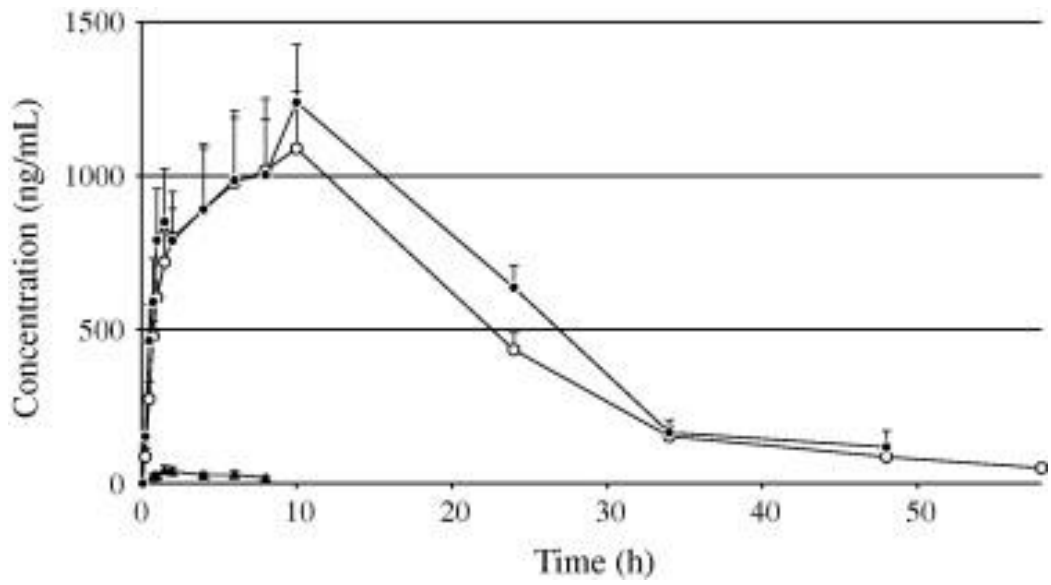


Fig. 2. Observed mean concentrations of tepoxalin in fasting horses (N), and its active metabolite (RWJ-20142) in fasting (d) and fed horses (s), following single oral administration of 10 mg/kg tepoxalin (n = 6). Bars represent standard error of the mean.

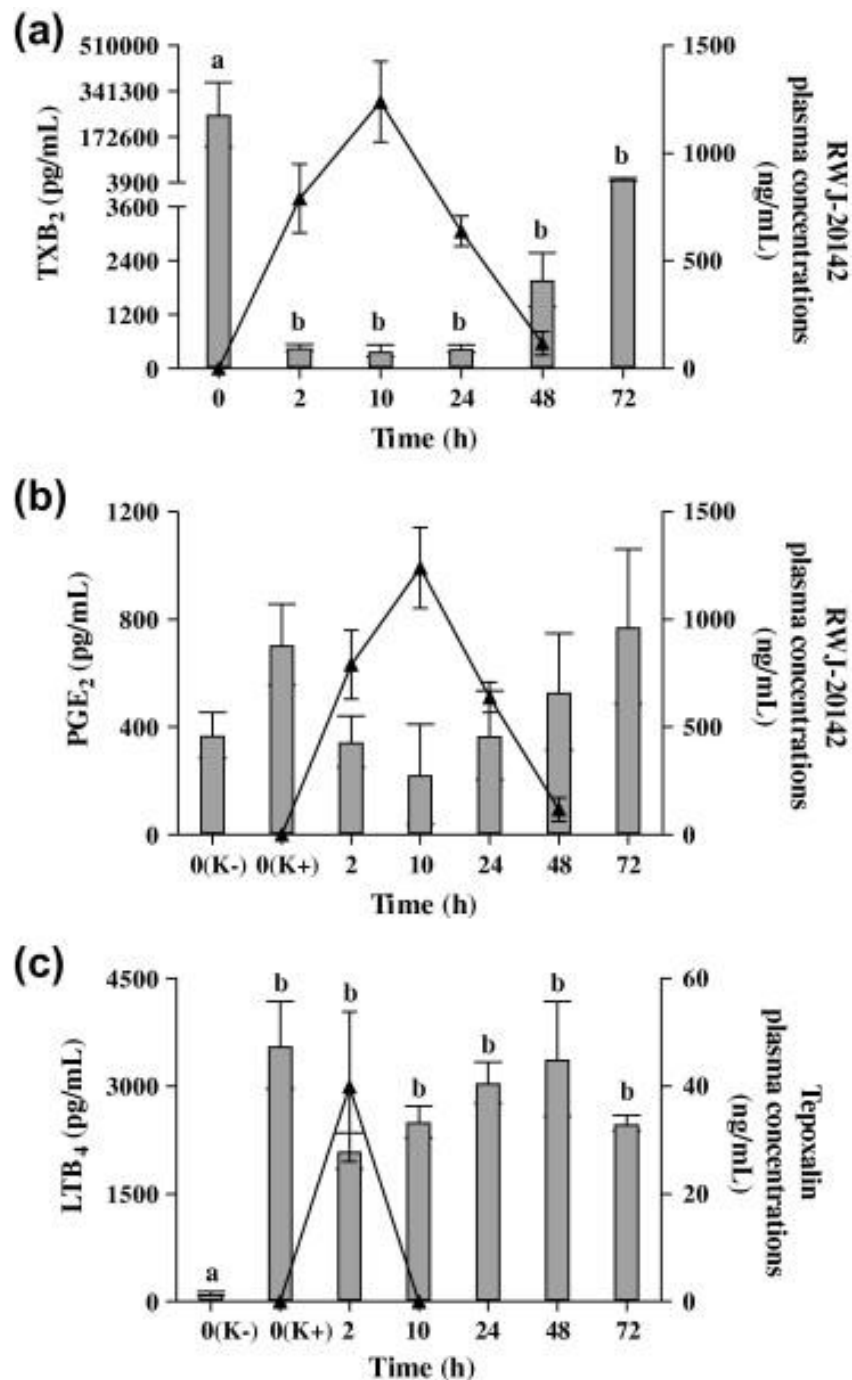


Fig. 3. Mean \pm SEM ($n = 3$) concentrations of thromboxane B2 (TXB₂; a), prostaglandin E2 (PGE₂; b) and leukotriene B4 (LTB₄; c) vs. RWJ-20142 or tepoxalin plasma concentrations in fasting equine whole blood before and after single oral administration of tepoxalin (10 mg/kg). Within time intervals, repeated-measures one-way ANOVA found values significantly different for TXB₂ ($P < 0.01$; $P < 0.05$) and for LTB₄ ($P < 0.001$; $P < 0.01$). K- = negative control; K+ = positive control.

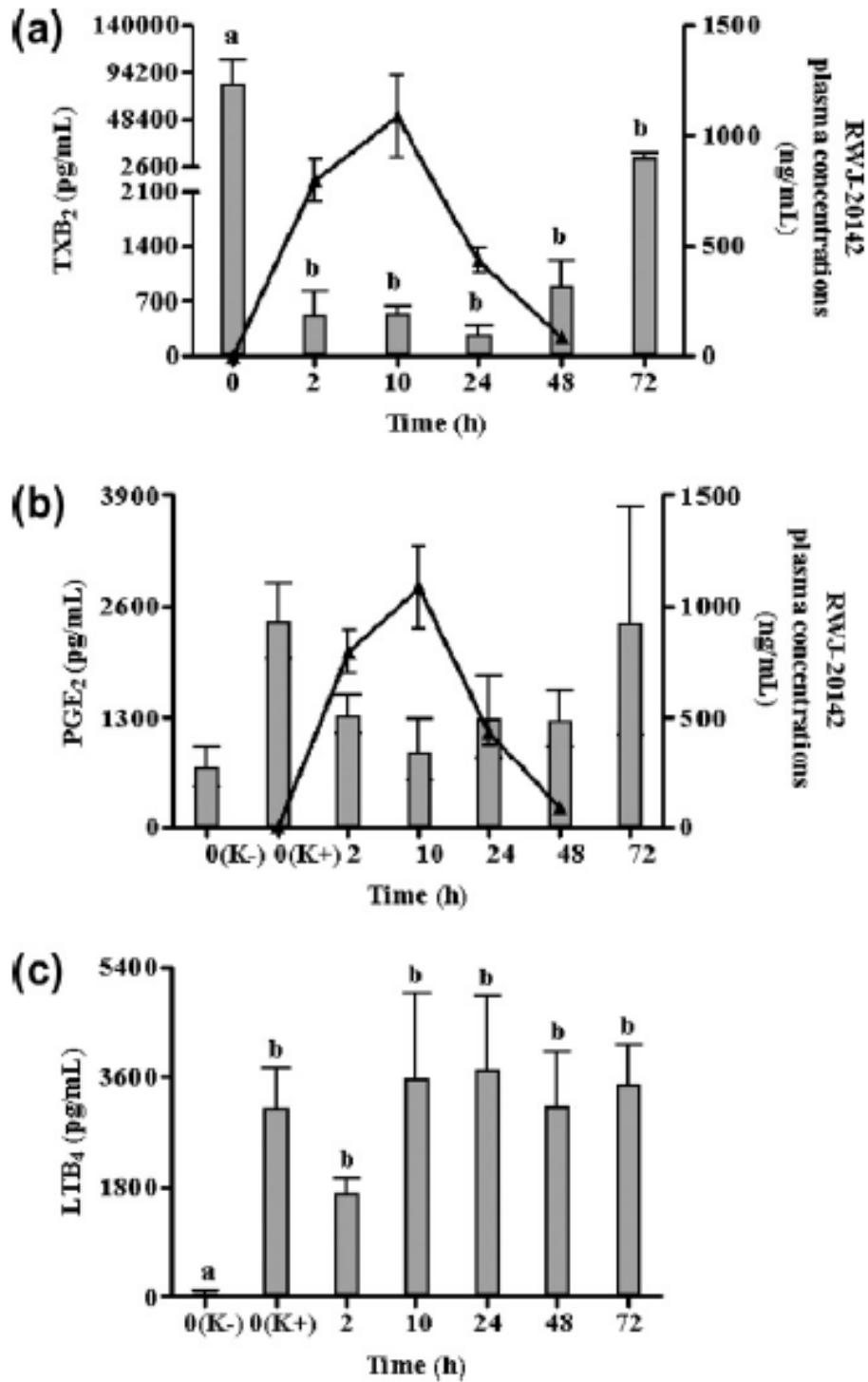


Fig. 4. Mean \pm SEM ($n = 3$) concentrations of thromboxane B₂ (TXB₂; a), prostaglandin E₂ (PGE₂; b) vs. RWJ-20142 plasma concentrations, and leukotriene B₄ (LTB₄; c) in fed equine whole blood before and after single oral administration of tepoxalin (10 mg/kg). Within time intervals, repeated-measures one-way ANOVA found values significantly different for TXB₂ ($P < 0.001$) and for LTB₄ ($P < 0.01$; $P < 0.05$). K- = negative control; K+ = positive control.

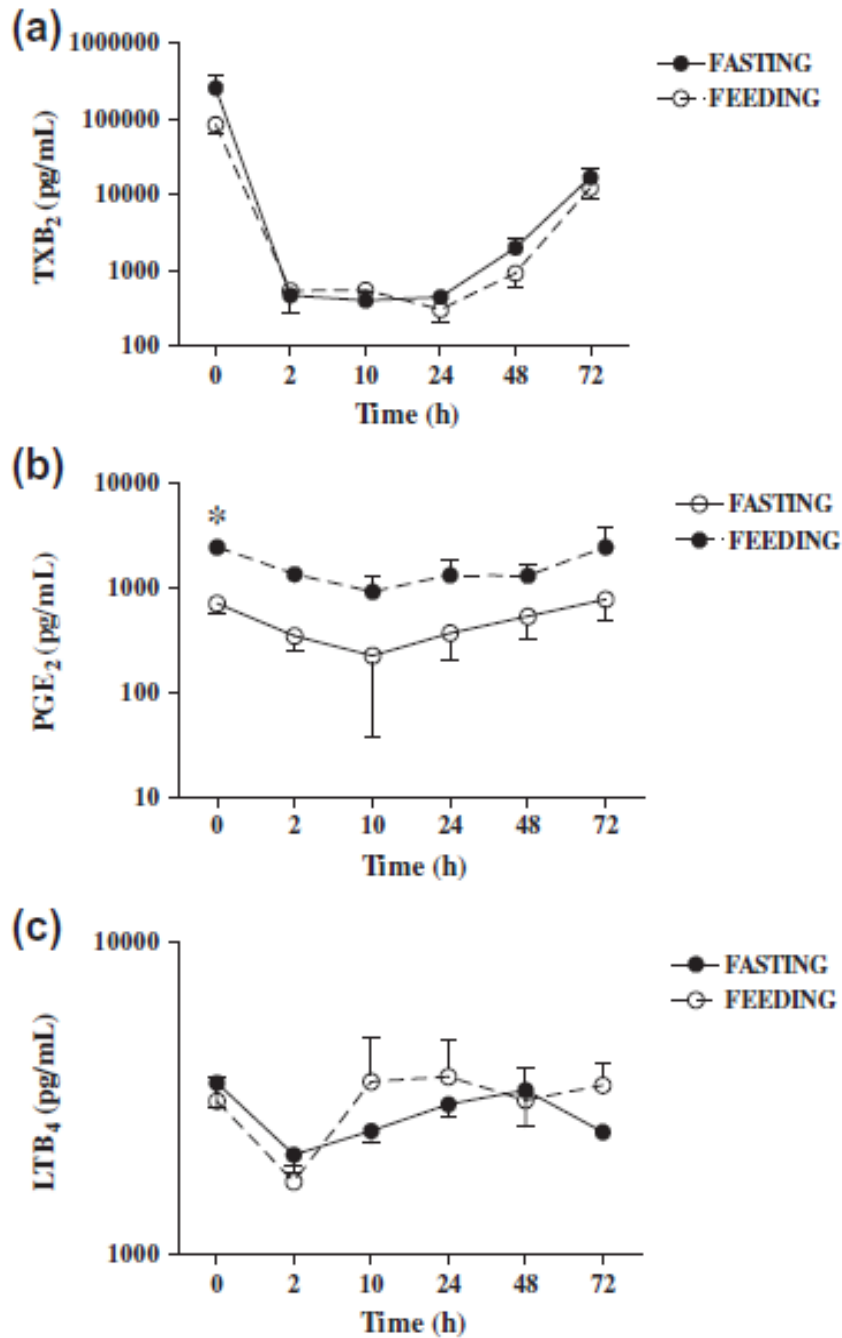


Fig. 5. Effect of feeding and fasting status on thromboxane B2 (TXB₂; a), prostaglandin E2 (PGE₂; b) and leukotriene B4 (LTB₄; c) production in equine whole blood (n = 3) before and after oral single administration of tepoxalin (10 mg/kg). No interactions were seen between TXB₂ and LTB₄ levels and the feeding/fasting status (P = not significant), whereas interactions were seen between PGE₂ levels and the feeding/fasting status before tepoxalin administration (*P < 0.05). Statistical analysis: two-way ANOVA.

Parameter	Tepoxalin		RWJ-20142			
	Fasting		Fasting		Fed	
	Mean	±SEM	Mean	±SEM	Mean	±SEM
r^2	0.98 ¹	0.01	0.93	0.03	0.98	0.01
λ_z (1/h)	0.40 ¹	0.1	0.1	0.01	0.07	0.01
$T_{1/2\lambda_z}$ (h)	2.78 ¹	0.99	6.3 ^a	0.1	8.8 ^a	1.0
Tmax (h)	1.5 ²	0.2	9.6	0.4	7.7	1.3
Cmax (ng/mL)	96.8 ²	55.0	1250	187	1197	198
VzF (mL/kg)	153.1	50.1	Nd	Nd	Nd	Nd
CL (mL/h/kg)	50.1 ¹	17.4	Nd	Nd	Nd	Nd
AUC _{last} (h ng/mL)	190 ¹	45.3	27,564	3307	24,553	3286
AUMC _{last} (h ² ng/mL)	640 ¹	237.7	403,410	38,543	317,495	48,179
MRT _{last} (h)	3.0 ¹	0.6	15.2	1.0	15.1	1.5

Table 1

Pharmacokinetic parameters of tepoxalin and RWJ-20142 following oral administrations of 10 mg/kg of tepoxalin in fasting and fed horses (n = 6 except where indicated).

r^2 , correlation coefficient; λ_z , terminal phase rate constant; $T_{1/2\lambda_z}$, terminal half-life; Tmax, time of peak; Cmax, peak plasma concentration; VzF, volume of distribution based on the terminal phase; CL, clearance of the terminal phase; AUC_{last}, area under the plasma concentration–time curve; AUMC_{last}, area under the first moment curve; MRT_{last}, mean resident time. Data originated from four¹ and five² horses. Nd = not detected.

^a Value significantly different (P < 0.05) between fasting and fed groups.

Time (h)	Inhibition (%)					
	TXB ₂		PGE ₂		LTB ₄	
	Fasting	Fed	Fasting	Fed	Fasting	Fed
2	99.7 ± 0.1 ^a	99.4 ± 0.2 ^a	48.4 ± 17.7	49.4 ± 3.6	37.5 ± 2.9 ^a	43.4 ± 4.7
10	99.6 ± 0.2 ^a	99.2 ± 0.3 ^a	68.9 ± 26.6	65.1 ± 8.0	23.5 ± 11.1 ^{ab}	-8.3 ± 20.2
24	99.7 ± 0.1 ^a	99.6 ± 0.1 ^a	46.2 ± 23.3	48.9 ± 11.3	-3.1 ± 9.4 ^b	-14.6 ± 13.4
48	98.9 ± 0.4 ^a	98.9 ± 0.3 ^a	27.9 ± 15.1	45.8 ± 14.3	2.0 ± 11.2 ^{ab}	1.8 ± 8.8
72	90.4 ± 4.3 ^b	85.8 ± 1.1 ^b	-5.7 ± 28.0	11.0 ± 34.8	11.3 ± 1.1 ^{ab}	-15.4 ± 18.2

Table 2

Mean ± SEM (n = 3) of percentage (%) inhibition of thromboxane B2 (TXB₂), prostaglandin E2 (PGE₂) and leukotriene B4 (LTB₄) in fasting/fed equine whole blood before and after single oral administration of tepoxalin (10 mg/kg).

Repeated-measures one-way ANOVA revealed significant differences in % inhibition of TXB₂ of fasting (^a vs. ^b, P < 0.05) and fed horses (^a vs. ^b, P < 0.001), and LTB₄ of fasting horses (^a vs. ^b, P < 0.05). No statistical differences were detected between fasting and fed groups.