Pharmacokinetic parameters and milk concentrations of ketoprofen after administration as a single intravenous bolus dose to lactating goats

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Six clinically normal lactating does were administered ketoprofen (2.2 mg/kg intravenously (i. v.)). Blood and milk samples were collected prior to and for 24 h after drug administration. Drug concentrations in serum and milk were determined by high performance liquid chromatography. Pharmacokinetic parameters from each goat were combined to obtain mean estimates (mean ± SD) of half-life of elimination ($t_{½\beta}$) of 0.32 ± 0.14 h, systemic clearance (Cl) of 0.74 ± 0.12 L/kg·h, and volume of distribution at steady state (V_{ss}) of 0.23 ± 0.051 L/kg. In milk, ketoprofen was unmeasurable by the method employed (level of detection 25 ng/mL) for all samples.

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INTRODUCTION

Non-steroidal antiinflammatory drugs (NSAIDs) are not approved for use in goats by the United States Food and Drug Administration (FDA). However, clinical conditions exist for which the use of a NSAID as an adjunct therapy may be indicated and beneficial. This may include conditions such as coliform mastitis, arthritis, laminitis, endotoxaemia, and other inflammatory disorders (Anderson *et al.*, 1991; Smith & Sherman, 1994). Ketoprofen, a propionic acid derivative, is a NSAID which is currently approved for alleviation of inflammation and pain in the horse. Ketoprofen has been reported to be a cyclooxygenase and lipoxygenase inhibitor (Kantor, 1986); however, it may not possess 5-lipoxygenase inhibitory action in calves (Landoni *et al.*, 1995a) or horses (Landoni & Lees, 1995). Ketoprofen and other NSAIDs have been reported to inhibit bradykinin-induced oedema in calves (Landoni *et al.*, 1995b).

Ketoprofen was less toxic to the kidney and gastrointestinal tract in horses than either phenylbutazone or flunixin meglumine (MacAllister *et al.*, 1993). Ketoprofen has been reported to reduce faecal output in calves with *E. coli* enterotoxaemia-induced diarrhea (Roussel *et al.*, 1993). Cattle with clinical mastitis had significantly improved recovery when ketoprofen was used as adjunctive therapy (Shpigel *et al.*, 1994). Pharmacokinetics of ketoprofen have been reported following a single injection in calves (Landoni *et al.*, 1995a), lactating dairy cattle (DeGraves *et al.*, 1996), humans (Debruyne *et al.*, 1987; Jamali & Brocks, 1990), horses (Owens *et al.*, 1995), dogs (Schmitt & Guentert, 1990), and following multiple intravenous doses in mares (Sams *et al.*, 1995).

The purpose of the study reported here was to determine the pharmacokinetics and disposition of ketoprofen in milk after intravenous (i. v.) administration in lactating goats.

MATERIALS AND METHODS

Goats

Six clinically normal, lactating Toggenburg does were used (body weight, 49–59 kg). Ages ranged from 1 to 4 years. All goats received a general physical examination and were weighed the day prior to drug administration. Udder health was determined by use of the California Mastitis Test (CMT) and udder palpation. Goats were milked by machine twice daily. They were kept on pasture during the day and confined to indoor group pens at night. Feed consisted of pasture, alfalfa hay, and a grain concentrate. During the 24 h sampling period, a study goat was sole occupant of a pen, fed grain concentrate, and provided alfalfa hay, water, and trace mineral salt *ad libitum*. The study goat was milked with the herd.

Experimental protocol

The project was reviewed and approved by North Carolina State University's Institutional Animal Care and Use Committee. Ketoprofen (100 mg/mL; Fort Dodge Laboratories, Fort Dodge, IA, USA) was administered as a single intravenous bolus at a

dosage of 2.2 mg/kg body weight (BW) via a catheter in the right jugular vein. The catheter was flushed with 5 mL of saline and removed after drug delivery. Goats received ketoprofen immediately following a morning milking. After drug administration, 10 mL blood samples were collected from a catheter in the left jugular vein into glass tubes. Catheters were flushed with heparinized saline between samples. Blood samples were collected prior to, and at 5, 10, 20, 30, 45, 60, 75, 90, 120, 150, 180, 210 min, and 4, 6, 8, 10, 12, 24 h following administration. Blood samples were placed on ice, allowed to clot, centrifuged (1400 ***g*** for 10 min) to harvest serum, and serum stored at −75°C until assayed.

A composite sample of milk, 100 mL, was collected prior to and at each milking following drug administration. Milk was centrifuged (1200 ***g*** for 5 min), milk fat removed, and the skim milk was stored at −75°C until assayed.

Analytical method for ketoprofen

A liquid chromatographic (LC) method was developed for the determination of ketoprofen in caprine serum and milk. Aliquots (500 μL) of serum or milk were diluted with equal volume of acetonitrile-water (v:v), centrifuged (5000 ***g***) for 5 min, and the colourless supernatant was ready for injection. For goat serum, the LC separations were performed using a mobile phase consisting of 25% acetonitrile in water and 20% methanol in 0.01 M dipotassium phosphate (K_2HPO_4) (50:50, v:v) of pH 4, adjusted with 80% phosphoric acid. For goat milk, the LC separations were performed using a mobile phase consisting of 20% acetonitrile in water and 20% methanol in 0.025 M potassium phosphate (KH_2PO_4) (50:50, v:v) of pH 6, adjusted with 80% phosphoric acid. The mobile phase flow rate was 1.5 mL/min giving a 6–7 min retention time and 1.0 mL/min giving a 5–6 min retention time for ketoprofen on a Nova-Pak 4-μm, C18 column, 150 mm × 3.9 mm I.D. (Waters Corporation, Milford, MA) for serum and milk, respectively. The column effluent was analysed in the wavelength range 200–320 nm using the photodiode array (PDA) detector. The chromatograms were acquired at max 257 nm. Recoveries of ketoprofen from caprine serum spiked with 5000, 500 and 50 ng/mL were 90, 90 and 70%, with coefficients of variation of 3.5, 8.2 and 25.0%, respectively ($n = 5$). Figure 1 (a) shows a chromatogram for an unfortified caprine serum sample, while Fig. 1 (b) shows a caprine serum sample spiked with ketoprofen at 50 ng/mL. The limit of detection for the determination of ketoprofen in caprine serum, defined as the analyte concentration yielding a peak approximately three times the noise level, was about 6 ng/mL, using an injection volume of 50 μL. Recovery of ketoprofen from caprine milk spiked with 250 ng/mL was 100%, with a coefficient of variation of 13% ($n = 5$). Figure 2 shows a chromatogram of caprine milk ultrafiltrate fortified with ketoprofen at 250 ng/mL. The limit of detection was estimated to be ≈ 25 ng/mL, using an injection volume of 100 μL.

Pharmacokinetic analysis

The ketoprofen serum concentration-time profiles for each goat were analysed separately using an exponential stripping program (RSTRIP; MicroMath Scientific Software, Salt Lake City, UT, USA). Selection of the appropriate compartment model was based upon statistical criteria that assessed the improvement in fit as the model increased in complexity. Parameters obtained directly from the software were area under the serum concentration-time curve (*AUC*) for zero to infinite time, mean residence time (*MRT*), half-life of distribution ($t_{½\alpha}$), distribution rate constant (α), extrapolated zero-time intercept of the distribution phase (A), half-life of elimination ($t_{½\beta}$), elimination rate constant (β), and extrapolated zero-time intercept of the elimination phase (B). Systemic clearance (*Cl*) for ketoprofen was calculated as $Cl = D/AUC$, where D is the dose administered. The area under the first moment curve (*AUMC*) was derived from $AUMC = AUC \times MRT$. Apparent volume of distribution at steady state (V_{ss}) was calculated as $V_{ss} = Cl \times MRT$. The volume of distribution of the central compartment (V_c) was calculated as $V_c = D/(A + B)$.

The pharmacokinetic parameters from individual goats were combined to derive mean pharmacokinetic parameters (Table 1).

RESULTS

No adverse reactions were observed following i. v. administration of ketoprofen. Pharmacokinetic parameters (mean ± SD) for ketoprofen are summarized in Table 1. Serum concentration-time curve after i. v. administration was biphasic and followed first-order kinetics (Fig. 3). Ketoprofen disposition was best described by a biexponential equation of the form $C_{(t)} = Ae^{-\alpha t} + Be^{-\beta t}$, where $C_{(t)}$ is the drug concentration at time (t), A was 10.87 ± 1.58 μg/mL, B was 3.88 ± 3.17 μg/mL, and e is the base of the natural logarithm.

The concentration of ketoprofen in milk, for all samples, was below the level of detection (25 ng/mL).

DISCUSSION

The finding of a 2-compartment model for ketoprofen was similar to reports in cattle (DeGraves *et al.*, 1996), horses (Benoit *et al.*, 1992; Owens *et al.*, 1995), and humans (Debruyne *et al.*, 1987). The initial distribution phase was very rapid, $t_{½\alpha} = 0.08 \pm 0.05$ h; the early rapid decline of the serum concentration-time curve is primarily caused by distribution of the drug from the central, highly perfused compartment to tissues. Studies in cattle (DeGraves *et al.*, 1996), horses (Owens *et al.*, 1995) and humans (Debruyne *et al.*, 1987) have also reported very rapid half-lives of distribution (Table 2).

The harmonic mean for half-life of elimination ($t_{½\beta} = 0.32 \pm 0.14$ h), was shorter than $t_{½\beta}$ of ketoprofen in either cows (DeGraves *et al.*, 1996), horses (Owens *et al.*, 1995; Sams *et al.*, 1995), or humans (Debruyne *et al.*, 1987), but similar to calves (Landoni *et al.*, 1995a) (Table 2). A mean clearance (*Cl*) of 0.74 ± 0.12 L/kg· h was larger than previously reported for cows (DeGraves *et al.*, 1996), horses (Owens *et al.*, 1995) or humans (Debruyne *et al.*, 1987; Jamali & Brocks,

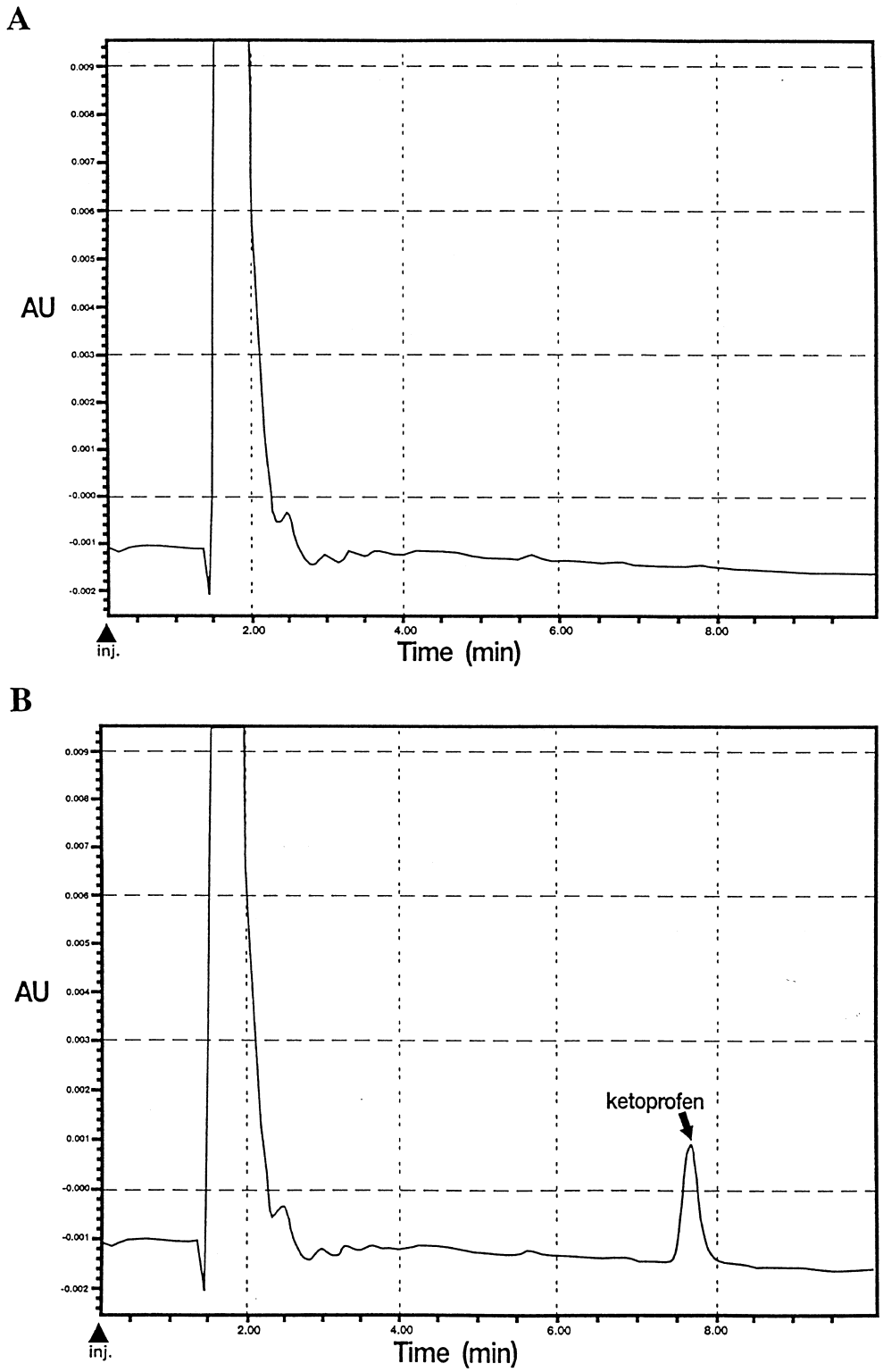

Fig. 1. (A) LC-UV PDA chromatogram (0–10 min) of an unfortified caprine serum sample acquired at 257 nm (λ_{max} for ketoprofen). Injection volume was 50 μL. (B) LC-UV PDA chromatogram (0–10 min) of a caprine serum sample spiked with ketoprofen at 50 ng/mL acquired at 257 nm (λ_{max} for ketoprofen). Injection volume was 50 μL.

1990) (Table 2). The observation of a shorter $t_{½\beta}$ and larger *Cl* for the goat compared to that reported in other species was not unexpected. An allometric relationship exists for physiological functions, in particular hepatic blood flow, correlated with body weight across different species (Adolph, 1949). By applying principles of allometry to pharmacokinetic parameters (Riviere

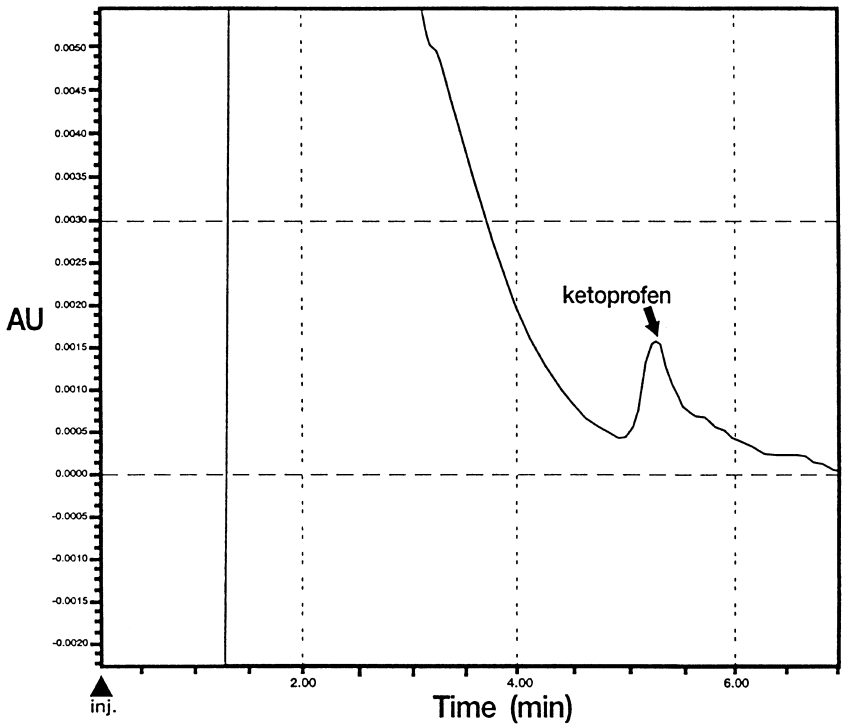

Fig. 2. LC-UV PDA chromatogram (0–7 min) of a caprine milk sample fortified at 250 ng/mL ketoprofen acquired at 257 nm (λ_{max} for ketoprofen). Injection volume was 100 μL.

et al., 1997), the finding of a larger clearance for the species with the smaller body weight may be expected. In humans, the hepatic microsomal enzyme glucuronyltransferase metabolizes ketoprofen to acyl-glucuronide conjugates (Jamali & Brocks, 1990); however, the metabolic elimination of ketoprofen in goats has not been studied. There is some evidence that goats have higher glucuronyltransferase activity than cattle (Short *et al.*, 1988), but the effect of this finding on ketoprofen biotransformation is unknown. Two other metabolic pathways for ketoprofen have been identified in the horse: reduction to a benzhydrolic metabolite and oxidation of the aromatic ring (Benoit *et al.*, 1992; Sams *et al.*, 1995); however, their presence in the goat has not been examined.

Table 1. Ketoprofen pharmacokinetic parameters following a single i. v. bolus administration of the drug (2.2 mg/kg of body weight) in 6 clinically normal lactating Toggenburg goats

Parameter	Units	Mean ± SD	Range
$t_{½\alpha}$	h	0.08 ± 0.05	0.04–0.17
A	mg/mL	10.87 ± 1.58	8.56–12.87
α	h^{-1}	8.92 ± 4.69	4.08–17.34
$t_{½\beta}$	h	0.32 ± 0.14	0.23–0.59
B	μg/mL	3.88 ± 3.17	3.80–9.68
β	h^{-1}	2.14 ± 0.70	1.14–3.00
$C_{(o)}$	μg/mL	14.75 ± 4.75	8.94–21.88
V_{ss}	L/kg	0.23 ± 0.05	0.15–0.35
V_c	L/kg	0.16 ± 0.05	0.11–0.20
Cl	L/kg · h	0.74 ± 0.12	0.56–0.90
AUC	μg · h/mL	3.05 ± 0.52	2.43–3.92
MRT	h	0.31 ± 0.06	0.25–0.42
AUMC	μg · h^2/mL	0.94 ± 0.03	0.67–1.40

Half-life reported as harmonic mean, $t_{½\alpha}$ = half-life of distribution, A = extrapolated zero–time intercept of the distribution phase, α = distribution rate constant, $t_{½\beta}$ = half-life of elimination, B = extrapolated zero–time intercept of the elimination phase, β = elimination rate constant, $C_{(o)}$ = initial serum concentration, V_{SS} = apparent volume of distribution at steady state, V_c = volume of distribution of the central compartment, *Cl* = systemic clearance, *AUC* = area under the serum concentration–time curve from zero to infinity, *MRT* = mean residence time, *AUMC* = area under the first moment curve.

The V_{ss} was 0.23 ± 0.05 L /kg, which is approximately equal to extracellular fluid volume (200 mL/kg). This is expected based upon the high degree of plasma protein binding of NSAIDs reported in other species (Kantor, 1986; Satterwhite & Boudinot, 1992; Owens*et al.*, 1995) (Table 2) and the high percentage of ionization at physiologic pH (pKa of ketoprofen is 5.02) (Schmitt & Guentert, 1990).

Landoni *et al.* (1995a) measured serum thromboxane and inflammatory exudate prostaglandin E_2 synthesis inhibition after ketoprofen administration up to 8 and 12 h, respectively. Inhibition persisted after ketoprofen concentrations in serum were below detection limit. Another investigator reported a similar finding of antiinflammatory action lasting longer than measurable serum concentrations (Sams *et al.*, 1995). These studies suggest that serum concentrations may not correlate with the antiinflammatory activity of ketoprofen. The dosage of 2.2 mg/kg BW used in this study is the labelled dosage for horses. Dosages of 3–6 mg/kg BW have been efficacious in cattle (Roussel *et al.*, 1993; Shpigel *et al.*, 1994; Landoni *et al.*, 1995a); however, the effective antiinflammatory or analgesic serum concentration of ketoprofen for goats has not been reported.

Ketoprofen in milk (first milking following drug administration, ≈ 8 h post injection) was below the limit of detection (25 ng/mL). This was similar to a study in cattle which reported detectable (lower limit = 27 ng/mL) though not quantifiable levels (lower limit = 90 ng/mL) of ketoprofen in milk up to 2 h

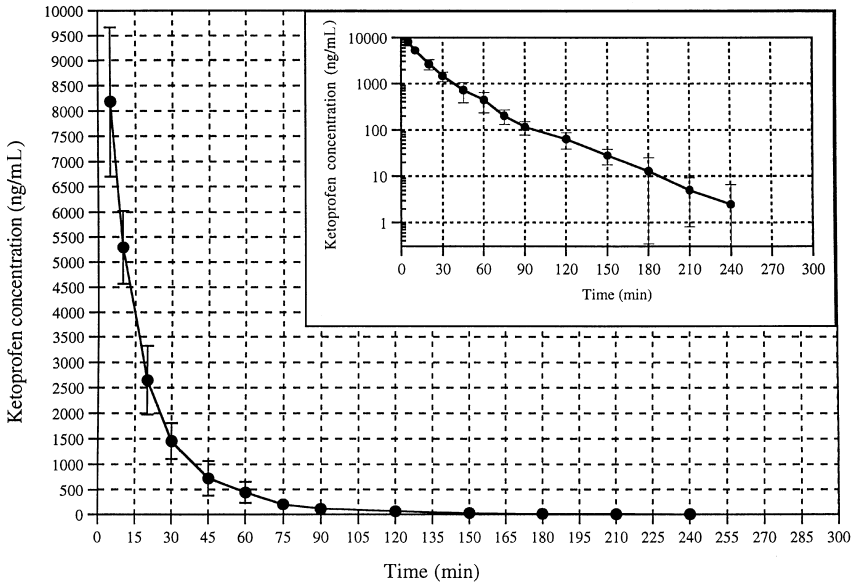

Fig. 3. Serum concentration-time curve (mean ± SD, $n = 6$) of ketoprofen administered as a single intravenous bolus (2.2 mg/kg). Insert is the semi logarithmic plot.

Table 2. Pharmacokinetic parameters of ketoprofen reported for various species

	$t_{½\alpha}$ (h)	$t_{½\beta}$ (h)	*Cl* (L/kg·h)	V_{SS} (L/kg)	Plasma Protein Binding (%)	References
Calves		0.42				Landoni *et al.* (1995a)
Cattle	0.07	0.48	0.17	0.10		DeGraves *et al.* (1996)
Horse	0.11	0.76–1.8	0.18	0.14	> 90	Owens *et al.* (1995); Sams *et al.* (1995)
Human	0.21	1.8	0.04–0.1	0.1–0.2	99	Kantor (1986); Debruyne *et al.* (1987) Jamali & Brocks (1990)
Rat					93–97	Satterwhite & Boudinot (1992)

$t_{½\alpha}$ = half-life of distribution, $t_{½\beta}$ = half-life of elimination, *Cl* = systemic clearance, V_{SS} = apparent volume of distribution at steady state.

after administration of 3.3 mg/kg i. v. (DeGraves *et al.*, 1996). Most drugs diffuse into and out of milk according to the pH-pKa ionization passive diffusion concept (Shore *et al.*, 1957; Ziv & Sulman, 1974). The free non ionized drug will diffuse bidirectionally, directly proportional to the concentration gradient until equilibrium of the non ionized fraction of the drug is achieved. Ketoprofen should have low concentrations in normal milk, due to the high degree of protein binding and high percentage of ionization in blood.

Pharmacodynamic and pharmacokinetic properties of individual enantiomers may differ in the chiral environment of the body. Ketoprofen is used as a racemic mixture of two optical isomers, R and S ketoprofen, of which the S enantiomer possesses nearly all the pharmacodynamic activity. Inter-and intraspecies variations in enantioselective disposition of ketoprofen have been reported (Landoni *et al.*, 1997). Individual enantiomeric disposition of ketoprofen was not examined in this study. The authors speculated that differences, if any, would be inconsequential based upon a previous report of no enantioselective differences in another ruminant species (cattle) (Landoni *et al.*, 1995a). Furthermore, determination of either enantiomer in milk would constitute an unacceptable drug residue. Irrespective of enantiomeric differences that may exist in disposition, no ketoprofen could be detected in milk.

The goal of this trial was to establish pharmacokinetic parameters and to determine ketoprofen disposition in the milk following i. v. administration. Due to ketoprofen's short half-life and physicochemical properties, ketoprofen may be of benefit in the acute treatment of inflammatory disorders and pose minimum potential to contaminate milk in lactating goats.

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