

Department of Equine and Small Animal Medicine  
University of Helsinki  
Finland

# **Pharmacology of ketoprofen administered orally to pigs: an experimental and clinical study**

Katja Mustonen

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Veterinary Medicine of the University of Helsinki, for public examination in Walter Hall, Agnes Sjöberginkatu 2, Helsinki on 30<sup>th</sup> November 2012, at 2:00 pm.

Helsinki 2012

**Supervised by**

Professor Outi Vainio

Department of Equine and Small Animal Medicine,  
Faculty of Veterinary Medicine,  
University of Helsinki, Finland

Docent Marja Raekallio, DVM, PhD

Department of Equine and Small Animal Medicine,  
Faculty of Veterinary Medicine,  
University of Helsinki, Finland

Professor Mari Heinonen

Department of Production Animal Medicine,  
Faculty of Veterinary Medicine,  
University of Helsinki, Finland

Professor Olli Peltoniemi

Department of Production Animal Medicine,  
Faculty of Veterinary Medicine,  
University of Helsinki, Finland

**Reviewed by**

Professor Birgit Ranheim

Norwegian School of Veterinary Science

Professor Leo van Leengoed

University of Utrecht, Netherlands

**Opponent**

Professor Dominiek Maes

University of Ghent, Belgium

ISBN 978-952-10-8377-8 (paperback)

ISBN 978-952-10-8378-5 (PDF)

Helsinki University Press

Helsinki 2012

# TABLE OF CONTENTS

TABLE OF CONTENTS	3
1. ABSTRACT	5
2. LIST OF ORIGINAL PUBLICATIONS	7
3. ABBREVIATIONS	8
4. INTRODUCTION	9
5. REVIEW OF THE LITERATURE	11
5.1 Ketoprofen	11
5.1.1 <i>Ketoprofen in pigs</i>	11
5.1.2 <i>Pharmacokinetics of ketoprofen in pigs</i>	12
5.1.3 <i>Enantiospecific pharmacokinetics of ketoprofen in other animal species</i>	13
5.1.4 <i>Pharmacodynamics of ketoprofen</i>	15
5.1.5 <i>Adverse events</i>	15
5.2 Endotoxemia in pigs	17
5.3 Non-infectious lameness in sows and gilts	18
6. AIMS OF THE STUDY	20
7. MATERIALS AND METHODS	21
7.1 Study design	21
7.2 Animals and clinical examinations	21
7.2.1 <i>Animals</i>	21
7.2.2 <i>Housing and management</i>	22
7.2.3 <i>Clinical examinations</i>	22
7.3 Treatments	23
7.3.1 <i>Racemic ketoprofen, dose rates and administration routes</i>	23
7.3.2 <i>Allocation to the treatment groups</i>	25

7.3.3. <i>Endotoxin challenge</i>	25
7.4 Blood sampling	25
7.5 Laboratory analysis	26
7.5.1. <i>Total ketoprofen plasma concentration analysis</i>	26
7.5.2. <i>Enantiospecific ketoprofen plasma concentration analysis</i>	27
7.5.3. <i>Analyses of thromboxane B<sub>2</sub> and haptoglobin plasma concentrations, and haematological parameters</i>	28
7.6 Pharmacokinetic parameters	28
7.7 Evaluation of efficacy in the treatment of lameness	29
7.8 Statistical analysis	29
8. RESULTS	31
8.1 Ketoprofen plasma concentrations	31
8.2 Pharmacokinetic parameters	35
8.3 Bioequivalence	36
8.4 Rectal temperatures and total clinical scores	36
8.5 TXB <sub>2</sub> plasma concentrations and laboratory analysis	37
8.6 Efficacy in the treatment of lameness	38
8.7 Adverse events	40
9. DISCUSSION	41
9.1 Pharmacokinetics of orally administered racemic ketoprofen	41
9.3 Efficacy in the treatment of endotoxemia	44
9.4 Efficacy in the treatment of lameness	45
9.4 Tolerance	46
10. CONCLUSIONS	47
11. ACKNOWLEDGEMENTS	48
12. REFERENCES	50
13. ORIGINAL ARTICLES	64

## 1. ABSTRACT

Ketoprofen is a non-steroidal anti-inflammatory drug belonging to the 2-arylpropionic acid group. It has been widely used in domestic animals because of its anti-inflammatory, antipyretic and analgesic actions. Ketoprofen is a chiral compound existing in two enantiomeric forms, S (+) and R (-) ketoprofen. Each enantiomer has different pharmacodynamic and pharmacokinetic properties. The commercial products in veterinary medicine are 50:50 racemic mixtures of both enantiomers. Ketoprofen undergoes unidirectional chiral inversion from the R- to the S-enantiomer, the extent of inversion being about 70% in pigs. The aims of this thesis research were to investigate the oral bioavailability, enantiospecific plasma concentrations and efficacy of racemic ketoprofen after oral administration in pigs.

Total plasma concentrations of ketoprofen were investigated in a randomized crossover design. Racemic ketoprofen was administered at 3 mg/kg and 6 mg/kg po, and 3 mg/kg im and iv. The bioavailability of oral ketoprofen was almost complete. Bioequivalence could not be detected between oral and intramuscular administration routes.

Plasma concentrations of S- and R-ketoprofen after oral (4 mg/kg) and intramuscular (3 mg/kg) routes of administration of racemic ketoprofen in pigs were determined in crossover design. S-ketoprofen was the predominant enantiomer in pig plasma after administration of the racemic mixture via both routes. The maximum plasma concentration of S-ketoprofen was more than twice as high as that of R-ketoprofen, and the terminal half-life was approximately three times greater for S-ketoprofen than R-ketoprofen with both administration routes. The mean ( $\pm$  SD) relative bioavailability (po compared to im) was  $83 \pm 20\%$  and  $63 \pm 23\%$  for S-ketoprofen and R-ketoprofen, respectively. Although some differences were detected in the plasma concentrations of ketoprofen enantiomers after different routes of administration, they are probably not relevant in clinical use. Thus, the pharmacological effects of racemic ketoprofen are considered to be comparable after intramuscular and oral routes of administration in growing pigs.

The efficacy of orally administered racemic ketoprofen in endotoxemia in pigs was assessed in an *E. coli* endotoxin challenge trial. The growing pigs were challenged intravenously with *E. coli* endotoxin and one hour after the challenge were treated with ketoprofen at dose rates of 0.5 mg/kg, 1 mg/kg, 2 mg/kg and 4 mg/kg or with tap water. The body temperature was measured and a total clinical score was calculated after assessing the general behaviour, respiratory rate and locomotion of the pigs. Thromboxane B<sub>2</sub> and ketoprofen concentrations were analysed from blood samples. Ketoprofen treatment significantly reduced the rectal temperature and total clinical scores, and lowered the blood thromboxane B<sub>2</sub> concentrations when compared to the control group. Ketoprofen plasma concentrations were lower than previously reported in healthy pigs after similar doses. Two mg/kg can be considered a sufficient oral dose of ketoprofen for treating endotoxemia in pigs, as increasing the dose above this did not further increase the effect.

Lameness is a common problem among sows and gilts. It often leads to poor animal welfare and economic losses because of unplanned culling. Locomotor disorders cause severe pain, and lame animals need to be treated accordingly. A field trial was conducted to examine the efficacy and compare two doses of oral administration of racemic ketoprofen in the treatment of clinical lameness caused by non-infectious musculoskeletal disorders in sows and gilts. Dose rates of 2 mg/kg and 4 mg/kg of oral ketoprofen were compared to placebo treatment over five consecutive days in a double-blinded study. Lameness was assessed with a five-grade scoring system prior to and on the last day of the treatment. This study demonstrated that oral ketoprofen was efficient in alleviating the signs of non-infectious lameness in sows and gilts. The rate of treatment success was 54.3% for the ketoprofen 4 mg/kg group, 53.2% for the ketoprofen 2 mg/kg group and 20.8% for the pigs in the placebo group. There was no difference in efficacy between the two ketoprofen doses. Oral ketoprofen appeared to be a practical way to alleviate pain and improve the welfare of lame sows. The findings support the use of ketoprofen at a dose rate of 2 mg/kg in treating locomotor disorders in pigs.

## 2. LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles, referred to in the text by their Roman numerals I–IV:

I Raekallio M.R., Mustonen K.M., Heinonen M.L., Peltoniemi O.A.T., Säkkinen M.S., Peltoniemi S.M., Honkavaara J.M., Vainio O.M. 2008. Evaluation of bioequivalence after oral, intramuscular, and intravenous administration of racemic ketoprofen in pigs. *American Journal of Veterinary Research* 69, 108-113.

II Mustonen K.M., Niemi A., Raekallio M.R., Heinonen M.L., Peltoniemi O.A.T., Palviainen M., Siven M.S., Peltoniemi M., Vainio O.M. 2012. Enantiospecific ketoprofen concentrations in plasma after oral and intramuscular administration in growing pigs. *Acta Veterinaria Scandinavia* 54, 55. doi:10.1186/1751-0147-54-55 (in press).

III Mustonen K., Banting A., Raekallio M., Heinonen M., Peltoniemi O., Vainio O. 2012. Dose response investigation of oral ketoprofen in pigs challenged with *E. coli* endotoxin. *Veterinary Record* 171, 70-73.

IV Mustonen K., Ala-Kurikka E., Orro T., Peltoniemi O., Raekallio M., Vainio O., Heinonen M. 2011. Oral ketoprofen is effective in the treatment of non-infectious lameness in sows. *The Veterinary Journal* 190, 55-59.

The original articles are reprinted with the kind permission of their copyright holders.

### 3. ABBREVIATIONS

AUC	area under the time-concentration curve
AUC <sub>0-∞</sub>	area under the time-concentration curve from time 0 to infinity
β	rate constant of the elimination phase
C <sub>max</sub>	maximum plasma concentration
CI	confidence interval
CL	total clearance
CMH	Cochran-Mantel-Haenszel
COX	cyclooxygenase
F	bioavailability
F <sub>rel</sub>	relative bioavailability
HPLC	high-performance liquid chromatography
IC <sub>50</sub>	the half maximal inhibitory concentration
im	intramuscular
ip	intraperitoneal
iv	intravenous
KTP	ketoprofen
LC-MS/MS	liquid chromatography-mass spectrometry
LOQ	limit of quantification
MAT	mean absorption time
MRT	mean residence time
NSAID	non-steroidal anti-inflammatory drug
PCV	packed cell volume
PDS	postpartum dysgalactia syndrome
PK/PD	pharmacokinetics/pharmacodynamics
po	per os
SD	standard deviation
SEM	standard error of the mean
T <sub>max</sub>	time to maximum plasma concentration
t <sub>1/2</sub>	elimination half-life
TXB <sub>2</sub>	thromboxane B <sub>2</sub>
V <sub>d</sub>	volume of distribution



## 4. INTRODUCTION

Animal welfare, legislation, environmental aspects, public health and food safety, as well as economics are among the issues to be considered when production animals are treated. Medication in veterinary medicine is strongly controlled by EU and national legislation. Veterinary medicinal products may only be administered to food producing animals if marketing authorization has been issued. In cases where there is no authorized medicinal product for a condition, a veterinary medicinal product authorized for use in another production animal species or for another condition in the same species may be used (2001/82/EEC). The use of some active substances is prohibited in animals because of their potential risk for public health (some antibiotics and substances for which safe minimal residue limits cannot be established) or animal welfare reasons (growth promoters) (96/22/EC). The use of medicinal products must not cause harm to the environment, either.

The treatment of production animals should be economically feasible. The withdrawal periods, the length of treatment and the cost of medication must be taken into consideration when animals are medicated. In some cases, euthanasia may be the preferred choice because of animal welfare or economics.

Pain and painful procedures performed in animals are the most common and emotive concerns among the public (Weary et al., 2006). Recognizing pain in animals is sometimes difficult and they might therefore be left untreated (Raekallio et al., 2003; Weary et al., 2006). Treatment may also cause pain, tissue irritation and side effects, which must be considered particularly when the duration of medication is prolonged (Bath, 1998; Pyörälä et al., 1999). Many infectious diseases cause a fever, which often leads to impaired appetite. In young animals, a reduced food intake or anorexia decreases growth rates, and in neonatal animals may lead to death. Antipyretic medication is therefore important as a supportive treatment.

Mastitis caused by Gram-negative bacteria, particularly *Escherichia coli*, is the most common cause of post-partum dysgalactia in sows (Wagner, 1982; Martineau et al., 1992; Reiner et al., 2009; Papadopoulos et al., 2010). The infection often induces a serious endotoxemic condition that leads to decreased milk production by the sow. Hypogalactia has the most important impact on production, as it will eventually lead to increased mortality among the piglets (Reiner et al., 2009). The treatment recommendation includes the use of NSAIDs with antimicrobials to inhibit bacterial growth and endotoxin production (Hirsch et al., 2003).

Lameness is a common problem among sows, and will often lead to premature culling or euthanasia of the lame animal (Kirk et al., 2005; Anil et al., 2009). It causes economic losses and ethical concerns in relation to animal welfare (Chagnon et al., 1991). In Finland, 9% of loose-housed sows have been found to be lame (Heinonen et al., 2006). The most common reasons for lameness are arthrosis or arthritis (Dewey et al., 1993; Kirk et al., 2005, Heinonen et al., 2006). Environmental aspects have a notable effect on the incidence of lameness at the farm level

(Kroneman et al., 1993; Heinonen et al., 2006). The improvement of environmental conditions does not eliminate the whole problem, and lame animals have to be treated properly. Musculoskeletal disorders may cause severe pain, and the relief of pain therefore has to be considered. The treatment of production animals has to be ethically acceptable and the need for pain relief should not be underestimated.

Ketoprofen is an NSAID belonging to the 2-arylpropionic acid group. It has been widely used in domestic animals because of its anti-inflammatory, antipyretic and analgesic actions. Ketoprofen is a chiral compound existing in two enantiomeric forms S (+) and R (-) ketoprofen. Each enantiomer has different pharmacodynamic and pharmacokinetic properties (Tucker and Lennard, 1990; Landoni et al., 1997). The commercial products in veterinary medicine are 50:50 racemic mixtures of both enantiomers. In the European Union, the need to set a maximum residue limit (MRL) for ketoprofen for bovine, porcine and equine animals has been assessed to protect public health, and it has been concluded that ketoprofen can be included in Annex II of the MRL regulation, i.e. ketoprofen is not subject to maximum residue limits (EC, 37/2010). The withdrawal time for meat is 2 to 4 days after intramuscular use, depending on the product, and 1 day after per oral use.

This dissertation concerns the pharmacokinetics of orally administered racemic ketoprofen in pigs and the efficacy of ketoprofen treatment in two common conditions for which the use of NSAIDs is indicated.

## 5. REVIEW OF THE LITERATURE

### 5.1 Ketoprofen

Ketoprofen, 2-(phenyl 3-benzoyl) propionic acid, is an NSAID belonging to the 2-arylpropionic acid group. It is a chiral compound existing in two enantiomeric forms, S (+) and R (-) ketoprofen. Ketoprofen undergoes unidirectional chiral inversion from the R- to the S-enantiomer in many animal species (Menzel et al., 1994; Aberg et al., 1995; Landoni and Lees, 1995b; Castro et al., 2000; Verde et al., 2001; Igarza et al., 2002; Arifah et al., 2003; Neirinckx et al., 2011). The mechanism involves stereoselective activation of R-ketoprofen by the formation of coenzyme A thioester, enzymatic epimerization of R-thioester to S-thioester and hydrolysis of S-thioester (Wechter et al., 1974; Hutt and Caldwell, 1983). The liver seems to have predominant role in the inversion process, although it takes place in various other organs as well, such as the intestine, lungs and kidneys (Cox et al., 1985; Mehvar and Jamali, 1988; Hall et al., 1992). The extent of inversion varies between species. Thioesterification, which is considered to be the limiting step of the chiral inversion process, is selective for the same aryl-2-propionic acid in various species (Soraci and Benoit, 1995).

#### 5.1.1 Ketoprofen in pigs

Intramuscular use of racemic ketoprofen is currently licensed for pigs in the European Union at a dose rate of 3 mg/kg for the treatment of porcine agalactia syndrome and respiratory tract infections (EMA, 1996). In 2009, an oral solution (Dinalgen 300 mg/ml, Laboratios Dr Esteve S.A., Barcelona, Spain) was granted marketing authorization in several EU countries for the treatment of fever and dyspnea associated with respiratory disease in fattening pigs at a dose rate of 1.5–3 mg/kg administered in drinking water (Irish Medicines Board, 2010). The maximum length of medication is three days for both administration routes. The withdrawal time for meat is 2 to 4 days after intramuscular use, mainly because of trace levels of residues at the injection site and depending on the product, and 1 day after per oral use.

Although ketoprofen has been indicated in pigs for its anti-inflammatory, analgesic and antipyretic activities for almost 20 years, there have been few reports on its clinical efficacy in pigs. Its potent antipyretic activity has been shown in pigs challenged with *Actinobacillus pleuropneumoniae* and *E. coli* endotoxin, and in field conditions in fattening pigs with respiratory diseases and sows with PDS (Arnaud and Consalvi, 1994; Swinkels et al., 1994; Salichs et al., 2012). In pigs challenged with *Actinobacillus pleuropneumoniae*, food consumption was also improved (Swinkels et al., 1994). Ketoprofen was administered orally at dose of 1.5 mg/kg in the *E. coli* endotoxin challenge study (Salichs et al., 2012) and intramuscularly at dose of 3 mg/kg in the other studies (Arnaud and Consalvi, 1994; Swinkels et al., 1994).

### 5.1.2 Pharmacokinetics of ketoprofen in pigs

Orally-administered racemic ketoprofen is absorbed well in pigs, and bioavailability is almost complete (Larsen et al., 1991; Neirinckx et al., 2011a). Bioavailability is also high after oral administration of R-ketoprofen alone (Neirinckx et al., 2011b). The absorption was reported to be nonstereoselective, as no presystemic inversion was present (Neirinckx et al., 2011b). Transcutaneous permeation of racemic ketoprofen is also reported to be nonstereoselective (Panus et al., 1999).

The predominant enantiomer in pig plasma is S-ketoprofen (Table 1) (Fosse et al., 2011a; Fosse et al., 2011b; Neirinckx et al., 2011a). The data of Fosse et al. (2011a) were obtained in neonatal animals (less than three weeks of age) and the data of Neirinckx et al. (2011a) in weaners (approximately 3 months of age). The pharmacokinetics of neonatal animals has some characteristic features. In general, the clearance is lower, the elimination half-life is longer and the volume of distribution larger in neonates compared to adults (Lees et al., 2004). The kinetics of ketoprofen is linear and the elimination half-life relatively short; approximately 0.5 h and 3.5 h for R-ketoprofen and S-ketoprofen, respectively (Fosse et al., 2011a; Fosse et al., 2011b; Neirinckx et al., 2011a). The maximum plasma concentration of R-ketoprofen is about the half of maximum plasma concentration of S-ketoprofen after oral and intramuscular administration of racemic ketoprofen (Fosse et al., 2011b; Neirinckx et al., 2011a). The inversion rate of R-ketoprofen to S-ketoprofen is rapid and high, approximately 70%, in pigs after po administration of R-ketoprofen (Neirinckx et al., 2011b). The inversion is mainly responsible for the faster clearance of R-ketoprofen after the administration of racemic ketoprofen. Stereoselectivity in protein or tissue binding, hepatic metabolism and renal excretion have not been studied in pigs and their influence cannot be completely excluded. The volume of distribution is low for both enantiomers with no differences between them (EMEA, 1996; Fosse et al., 2011a; Neirinckx et al., 2011a; Neirinckx et al., 2011b). Ketoprofen is highly bound to plasma protein and expected to distribute primarily to the extracellular fluids. Oral and intramuscular administration routes are likely to achieve smaller extravascular penetration than intravenous administration, which provides high initial plasma concentrations (Lees et al., 2004). Ketoprofen is converted into a carbonyl-reduced derivative, 2-(phenyl 3-alpha-hydroxybenzoyl) propionic acid in pigs (EMEA, 1996). After a single intramuscular injection of  $^{14}\text{C}$ -ketoprofen (3 mg/kg), 70% of the total radioactivity was excreted via urine within 96 hours, with most being excreted within 24 hours, and only 20% was recovered in faeces (EMEA, 1996). In urine, about 30% of the radioactivity was due to 2-(phenyl 3-alpha-hydroxybenzoyl) propionic acid, 12% to the parental compound and 45% to polar components (EMEA, 1996).

Table 1. Mean (95% CI) /  $\pm$  SD pharmacokinetic parameters after administration of racemic ketoprofen in pigs.

	Dose		
	6 mg/kg iv <sup>1</sup>	6 mg/kg im <sup>2</sup>	3 mg/kg po <sup>3</sup>
<b>S-ketoprofen</b>			
T <sub>1/2</sub> (h)	3.3 (2.8–3.7)	3.5 (3.5–3.6)	2.2 $\pm$ 0.4
MRT (h)	4.6 (3.9–5.5)		3.9 $\pm$ 1.0
AUC <sub>0-∞</sub> (μgh/mL)	88.9 (71.1–111.1)	76.6 (63.2–92.9)	31.3 $\pm$ 6.7
C <sub>max</sub> (μg/mL)	19.1 (17.3–21.1)	13.1 (11.9–14.5)	7.3 $\pm$ 1.6
T <sub>max</sub> (h)		0.68 (0.51–0.84)	0.6 $\pm$ 0.3
<b>R-ketoprofen</b>			
T <sub>1/2</sub> (h)	0.4 (0.3–0.5)	0.5 (0.5–0.6)	0.8 $\pm$ 0.2
MRT (h)	0.5 (0.4–0.6)		1.3 $\pm$ 0.3
AUC <sub>0-∞</sub> (μgh/mL)	7.1 (5.3–9.4)	7.5 (6.1–9.2)	4.3 $\pm$ 1.1
C <sub>max</sub> (μg/mL)	10.1 (8.2–12.5)	7.0 (5.9–8.3)	3.9 $\pm$ 1.1
T <sub>max</sub> (h)		0.3 (0.2–0.3)	0.3 $\pm$ 0.2

<sup>1</sup>Fosse et al., 2011a; <sup>2</sup>Fosse et al., 2011b; <sup>3</sup>Neirinckx et al., 2011a.

### 5.1.3. Enantiospecific pharmacokinetics of ketoprofen in other animal species

The enantiospecific pharmacokinetics of ketoprofen have been studied in several production animal species other than pigs, including llamas (Navarre et al., 2001), calves (Landoni et al., 1995b; Landoni and Lees, 1995b), dairy cattle (Igarza et al., 2004), horses (Jausaud et al., 1993; Landoni and Lees, 1996; Verde et al., 2001), sheep (Arifah et al., 2001; Landoni et al., 1999) and goats (Arifah et al., 2003). In most animal species, such as rats (Foster and Jamali, 1988), dogs (Montoya et al., 2004), cats (Lees et al., 2003) and horses (Verde et al., 2001), S-ketoprofen is the predominant enantiomer in the plasma after administration of the racemic drug, whilst R-ketoprofen is predominant in sheep (Arifah et al., 2001), camels (Al Katheeri et al., 2000) and elephants (Hunter et al., 2003). The plasma concentrations of both enantiomers are equal in goats (Arifah et al., 2003), calves (Landoni et al., 1995b; Landoni and Lees, 1995b), llamas (Navarre et al., 2001) and humans (Sallustio et al., 1988). Plasma concentrations of both enantiomers have been reported to be higher in female camels than males, and stereoselectivity in the pharmacokinetics of racemic ketoprofen was more evident in females than males (Al Katheeri et al., 2000). Gender dependence of urinary excretion of conjugated S-ketoprofen and a faster total clearance of S-ketoprofen in males than in females has been reported in rats (Palylyk and Jamali, 1992), whereas in a human study the elimination rate constant for R-ketoprofen was higher in males than females (Rudy et al., 1998). The pharmacokinetic parameters of S- and R-ketoprofen in different large animal species after iv administration of racemic ketoprofen are presented in Table 2. The elimination half-life of ketoprofen enantiomers is relatively short in all animal species studied. Measurable concentrations are detected in exudate much longer than in plasma, which may be due to a high level of plasma protein binding and protein, with bound drug, leaking into exudate from plasma (Arifah et al., 2001; Arifah et al., 2003; Landoni et al., 1995b; Landoni et al., 1999).

Table 2. Mean  $\pm$  SEM (goat, horse, calf, sheep)/  $\pm$ SD (llama)/ range (camel, elephant) pharmacokinetic parameters for S- and R-ketoprofen after iv administration of racemic ketoprofen to different large animal species.

	Dose (mg/kg)	Parameter (unit)					
		S-ketoprofen			R-ketoprofen		
		$T_{1/2}$ (h)	MRT (h)	AUC <sub>0-∞</sub> ( $\mu$ gh/mL)	$T_{1/2}$ (h)	MRT (h)	AUC <sub>0-∞</sub> ( $\mu$ g /mL)
Goat <sup>1</sup>	3	1.79 $\pm$ 0.24	1.28 $\pm$ 0.2	5.15 $\pm$ 0.46	1.87 $\pm$ 0.28	1.36 $\pm$ 0.29	6.81 $\pm$ 0.66
Horse <sup>2</sup>	2.2	1.14 $\pm$ 0.18	0.93 $\pm$ 0.13	5.54 $\pm$ 0.98	1.87 $\pm$ 0.63	1.40 $\pm$ 0.51	3.13 $\pm$ 0.50
Camel <sup>3</sup> female	2	1.83 (1.67–2.26)	2.08 (1.79–2.39)	37.3 (31.8–52.9)	1.88 (1.42–2.34)	2.13 (1.80–2.96)	22.4 (13.5–29.7)
Camel <sup>3</sup> male	2	2.33 (1.52–3.83)	2.2 (1.54–3.24)	14.4 (11.0–19.3)	2.11 (1.50–4.20)	2.17 (1.70–4.04)	16 (12.9–22.4)
Elephant <sup>4</sup>	2.2	5 (3.7–7.6)	4.6 (3.2–5.8)	77.35 (51.26–132.46)	5.5 (4.7–7.3)	6.6 (4.4–8.1)	123.65 (86.67–194.81)
Calf <sup>5</sup>	3	0.42 $\pm$ 0.09	2.67 $\pm$ 0.79	9.94 $\pm$ 1.09	0.42 $\pm$ 0.08	1.69 $\pm$ 0.25	9.49 $\pm$ 0.84
Llama <sup>6</sup>	4.4	5.49 $\pm$ 1.27	7.72 $\pm$ 1.56	168.9 $\pm$ 22.4	5.41 $\pm$ 0.94	7.56 $\pm$ 1.13	176.4 $\pm$ 25.5
Sheep <sup>7</sup>	3	0.63 $\pm$ 0.05	0.64 $\pm$ 0.04	5.82 $\pm$ 0.45	0.63 $\pm$ 0.05	0.77 $\pm$ 0.1	10.54 $\pm$ 0.92

<sup>1</sup>Arifah et al., 2003; <sup>2</sup>Verde et al., 2001; <sup>3</sup>Al Katheeri et al., 2000; <sup>4</sup>Hunter et al., 2003; <sup>5</sup>Landoni et al., 1995b; <sup>6</sup>Navarre et al., 2001; <sup>7</sup>Arifah et al., 2001.

After oral administration of racemic ketoprofen, absorption is rapid but bioavailability varies between animal species. In the cat (Lees et al., 2003), dog (Schmitt and Guentert, 1990), elephant (Hunter et al., 2003) and rat (Jamali and Brocks, 1990), bioavailability is high, whereas in horses it is extremely low (Landoni and Lees, 1995c). Ketoprofen is metabolised by glucuronidation and mainly excreted in urine (Jamali and Brocks, 1990). Enterohepatic circulation of ketoprofen has been suggested in dogs and rats, which mainly eliminate ketoprofen into bile (Yasui et al., 1996; Granero and Amidon, 2008).

The inversion rate from R-ketoprofen to S-ketoprofen varies between species. In the dog, hamster and rat, the rate of inverted R-enantiomer was significantly above 50% (Aberg et al., 1995), whereas in the cat (Castro et al., 2000), calf (Landoni and Lees, 1995c), mouse (Aberg et al., 1995), gerbil (Aberg et al., 1995), rabbit (Aberg et al., 1995), monkey (Aberg et al., 1995), dairy cow (Igarza et al., 2002) and guinea pig (Aberg et al., 1995) the level of inversion was limited. In dairy cows, the percentage of inversion differed according to the physiological status, being higher in early lactation than during the dry period (Igarza et al., 2002). The extent of inversion is not affected by the dose rate (Menzel et al., 1994; Geisslinger et al., 1995). Administration of racemic ketoprofen instead of a pure enantiomer may have an influence on the enantiomeric plasma concentration ratio. In mice the S-ketoprofen/total ketoprofen ratio decreased after administration of racemic ketoprofen, although the inversion rate was above 40% after administration of pure R-

ketoprofen (Aberg et al., 1995). In calves no differences were detected between disposition curves of enantiomers after administration of racemic ketoprofen, however inversion rate of 30 % has been reported after administration of pure R-ketoprofen (Landoni et al., 1995b; Landoni and Lees, 1995b).

#### 5.1.4. *Pharmacodynamics of ketoprofen*

The primary mechanism of action of ketoprofen is the inhibition of COX, thereby blocking prostaglandin biosynthesis from arachidonic acid (Vane, 1971). COX exists in two isoforms, COX-1 and COX-2, both being targets of ketoprofen and other NSAIDs. Pro-inflammatory prostaglandins have an important role in inflammation by inducing fever and hyperalgesia, and mediating inflammatory swelling and vasodilatation, thus provoking the cardinal signs of inflammation: redness, swelling, heat, pain and loss of function (Diez-Dacal and Perez-Sala, 2010). They synergise with mediators such as histamine and bradykinin, which increases permeability. Pyrogenic prostaglandins are produced in the hypothalamus and released by bacterial pyrogen stimulation. Prostaglandins do not directly stimulate nociceptors, but they enhance nociceptive stimuli produced by other mediators, including serotonin, bradykinin and histamine (Lees et al., 2004). This phenomenon, which increases sensitivity to pain, is called hyperalgesia. Prostaglandins are also involved in the allodynia process, in which a stimulus that is not normally painful becomes painful in damaged tissue (Hersh et al., 2000). Ketoprofen also inhibits lipoxynase enzyme, which blocks bradykinin production (Lees et al., 2004). It crosses the blood-brain barrier and also has central analgesic activity (de Beaurepaire et al., 1990; Herrero et al., 1997; Diaz-Reval et al., 2004).

Ketoprofen inhibits both COX enzymes, although it is considered as a COX-1-selective drug (Brideau et al., 2001; Streppa et al., 2002). S-ketoprofen is regarded as a more potent COX inhibitor than R-ketoprofen (Cabre et al., 1998a; Cabre et al., 1998b). Inhibition of COX-2 is almost exclusively attributed to S-ketoprofen (Suesa et al., 1993).

Ketoprofen has central and peripheral activity, which is most probably independent of COX inhibition (Carabaza et al., 1996). R-ketoprofen might be responsible for this activity. It has been shown that R-ketoprofen has a more potent analgesic effect than S-ketoprofen (Ossipov et al., 2000; Pinardi et al., 2001). Intrathecal administration of R-ketoprofen produced a dose-dependent antiallodynic effect in rats, while S-ketoprofen did not (Ghezzi et al., 1998). PK/PD modelling of the mechanical nociceptive threshold in piglets with kaolin-induced inflammation gave a median potency ratio  $IC_{50R}/IC_{50S}$  of 0.06, indicating the more potent analgesic action of R-ketoprofen (Fosse et al., 2011b). The COX-independent action of R-ketoprofen has not yet been fully clarified.

#### 5.1.5. *Adverse events*

NSAIDs reduce pain and inflammation by blocking COX enzymes that are needed to produce prostaglandins and thromboxanes (Vane, 1971). The typical side effects of NSAIDs are also caused by this COX inhibition, although some adverse events are not explained by COX inhibition (Little

et al., 2007). COX-1, a house-keeping enzyme, is present in most cell types excluding erythrocytes and affects prostaglandins, which perform a range of physiological functions such as blood clotting, and reno- and gastroprotection (Miller, 2006; Little et al., 2007). COX-2, which is an inducible enzyme, is formed at inflammation sites and produces pro-inflammatory prostaglandins. It has been found to be present in many tissues, including the gastrointestinal tract as well as neural, reproductive and renal tissues, and also has physiological functions (Dinchuk et al., 1995; Miller, 2006; Little et al., 2007).

The most frequent side effect of NSAIDs is damage to the gastrointestinal mucosa, which is mostly caused by COX-1 inhibition and is independent of the administration route (Wallace, 1997). Clinical signs in gastrointestinal irritation and ulceration include depression, anorexia, reduced appetite, diarrhoea, vomiting and haematochezia and melena (Stanton and Bright, 1989; Hinton et al., 2002). Both COX isoforms are constitutively present in the kidneys (Miller, 2006), and inhibition of either COX-1 or COX-2 may therefore result in kidney failure (Lascelles et al., 2005). Dehydration, hypovolemia and geriatric age are predisposing factors for renal side effects (Khan et al., 1998). Hepatotoxicity may be related to the inhibition of either COX isoform (Carrillo-Jimenez and Nurnberger, 2000; Chitturi and George, 2002). Heart-related adverse effects in humans, such as heart failure, myocardial infarction and stroke, appear to be largely dependent on COX-2 inhibition (Grosser et al., 2010). COX-1 inhibits thromboxane production, which may lead to prolonged bleeding, whereas selective COX-2 inhibition may increase the risk of thrombogenesis by decreasing prostaglandin-derived vasodilatation and antiaggregation (Miller, 2006).

NSAIDs differ in their selectivity for COX-isoforms, i.e. how much they affect COX-1 relative to COX-2 (Lees et al., 2004). This selectivity does not affect the efficacy of the drug, but it does affect the prevalence of adverse events, as gastrointestinal irritation is the most frequent side effect (Lascelles et al., 2005). NSAIDs with long half-lives or a slow-release formulation as well as high dose rates are associated with a greater risk of upper gastrointestinal bleeding (Masso Gonzalez et al., 2010).

Reported adverse effects caused by ketoprofen administration at normal treatment dose levels include gastroentero irritation and ulcers, a prolonged bleeding and clotting time, renal disorders and photosensitivity (Cabre et al., 1998a; Jerussi et al., 1998; Narita et al., 2005; Luna et al., 2007; Devleeschouwer et al., 2008). Most reports of side effects have been from humans, dogs or small laboratory animals. In humans, the estimated relative risk for gastrointestinal bleeding or perforation after ketoprofen use is 5.4 times higher compared to non-use (Masso Gonzalez et al., 2010). In anaesthetized piglets, ketoprofen significantly decreased the urinary flow, glomerular filtration rate and renal blood flow (Junot et al., 2008). Patients with end-stage renal disease have been reported to display selective accumulation of S-ketoprofen after administration of racemic ketoprofen (Grubb et al., 1999). Administration of racemic ketoprofen in rats caused more gastric toxicity than administration of each enantiomer alone (Alarcon de la Lastra et al., 2002). As gastric toxicity caused by the racemate was more than additive, it was suggested that the remaining R-ketoprofen, which is not inverted, may enhance the gastric toxicity of S-ketoprofen when administered as a racemate (Cabre et al., 1998a; Alarcon de la Lastra et al., 2002).



Oral administration of indomethacin, aspirin and naproxen for 10 days caused gastroduodenal ulcers in pigs (Rainsford et al., 2003). Significant blood loss associated with the appearance of gastric ulcers was only evident in pigs administered aspirin. Indomethacin also produced ulcers in the caecum. Ulcers caused by NSAIDs are located on the glandular part of the stomach. Gastric ulcers affecting the mucosa of the oesophageal part of the stomach are common in pigs (Robertson et al., 2002; Swaby and Gregory, 2012). In the UK, 80% of pigs examined after slaughter had gastric ulcers and in 6.4% the ulcers were graded as severe (Swaby and Gregory, 2012). Ulceration of the *pars oesophagea* is a multifactorial condition and has several predisposing factors such as feeding, infections and stress (Doster, 2000). Clinically, it is impossible to distinguish between ulcers in the oesophageal and the glandular area.

A therapeutic dose of 3 mg/kg of racemic ketoprofen administered iv for 5 consecutive days was well tolerated in calves (Singh et al., 2009). Gastrointestinal side effects tended to occur in dogs administered ketoprofen for 30 days, although the lesions healed after administration ceased and were not therefore clinically important in healthy dogs (Narita et al., 2005). Horses showed no clinical side effects during ketoprofen administration at a dose of 2.2 mg/kg three times a day for twelve days (MacAllister et al., 1993). However, post-mortem examination revealed lesions in the glandular part of the stomach. In children, oral ketoprofen is well tolerated when administered up to three weeks (Kokki, 2010).

Ketoprofen is a weak acid and it may cause local irritation of the gastric mucosa when administered orally (Boothe, 2001). After intramuscular administration to cows it caused no clinical signs of pain, and only mild tissue irritation was detected (Pyörälä, 1999).

## 5.2 Endotoxemia in pigs

Postpartum dysgalactia syndrome, typically occurring within three days after farrowing, is an important cause of endotoxemia in pigs. Endotoxins are lipopolysaccharides that are major compounds of the outer membrane of Gram-negative bacteria. They are released from the cell wall during rapid bacterial growth and after lysis. Endotoxins have either a direct or indirect influence on multiple organs of the host animal. Their toxic effects include fever, dysfunctions in circulation and coagulation, hypoglycaemia and leucopenia, hypotension and vascular shock, and the activation of acute inflammatory reactions (Mayeux, 1997). Many of these effects are not actually caused by endotoxins directly, but by overreaction of host immune response. Endotoxins entering the circulation are transferred to cell-surface receptors, which primarily exist on mononuclear phagocytes. The sensitivity of cells to endotoxin increases and leads to the production of proinflammatory mediators. They are responsible for many pathophysiological reactions of endotoxemia. For this reason, the severity of the clinical illness is determined by the phagocyte response against endotoxins (Moore and Barton, 2003). Pigs challenged with *E. coli* endotoxin developed typical clinical signs such as an increased rectal temperature, anorexia, depression, lethargy, hypogalactia, agalactia, vomiting, diarrhoea, firmness and warmth of the udder, incoordination, coma and death (Elmore et al., 1978; Liggett et al., 1986). As endotoxins induce an increase in prostaglandin and thromboxane levels in the plasma in pigs, the beneficial effects of

NSAIDs in the treatment of induced endotoxic shock in pigs have been shown for several drugs, including indomethasin (Olson et al., 1985), flunixin meglumin (Olson et al., 1985), flubiprofen (Schrauwen et al., 1983), meloxicam (Friton et al., 2006), ketoprofen (Salichs et al., 2012) and ibuprofen (Fink et al., 1989). They increase survival and alleviate clinical signs caused by endotoxins.

The incidence of PDS varies between regions and individual herds, the average being approximately 10% (Backstrom, 1973; Threlfall and Martin, 1973; Backstrom et al., 1984; Thorup, 2000; Papadopoulos et al., 2010). Regardless of the organ systems primarily involved (mammary gland, uterus, urinary tract), the major pathophysiological aspect is bacterial endotoxemia, usually caused by *E. coli* (Reiner et al., 2009). The clinical signs are variable depending on the organs involved. Endotoxemia is usually associated with some or all of the following signs: mastitis, metritis, cystitis, anorexia, pyrexia and hypogalactia or agalactia (Hirsch et al., 2003; Reiner et al., 2009). Diseased sows seldom die unless severe endotoxemia occurs, but piglets of diseased sows are affected early during the course of the disease. Agalactia or hypolactia caused by endotoxins suppressing prolactin production may lead to starvation of newborn piglets (Smith and Wagner, 1984). The incidence of diarrhoea in piglets of PDS-treated sows increases (Thorup, 2000). Major economic losses are due to the increased death rates and decreased growth rates of the piglets. A lack of good hygiene, restricted movement and inadequate water and food intake are predisposing factors for coliform mastitis. The transferral of sows to farrowing unit at least one week before the expected farrowing date, allowance of sows to farrow naturally and frequent supervision of farrowing reduce the risk of PDS (Papadopoulos et al., 2010).

### 5.3 Non-infectious lameness in sows and gilts

Lameness is a common problem among sows and gilts. It often leads to poor animal welfare and economic losses because of unplanned removals (Chagnon et al., 1991). Lameness has a higher risk of removal from the herd than non-lame sows, and they are removed at a younger age than sows removed for other reasons (D'Allaire et al., 1987; Anil et al., 2009). Locomotor disorders have been the most common reason for culling sows in Denmark (Kirk et al., 2005) and Sweden (Engblom et al., 2008). In Finland, 9% of loose-housed sows and gilts were reported to be lame (Heinonen et al., 2006). The most common reason for lameness is osteochondrosis/osteoarthritis (Dewey et al., 1993; Heinonen et al., 2006). Gilts have a higher risk of becoming lame than older sows (Chagnon et al., 1991). Lameness is a clinical symptom of potential painful locomotor disorders, and pain may also lead to other problems. Food consumption decreases when sows are in pain, and milk production is therefore diminished and piglet mortality increases (Chen Cong Ying et al., 2008). A lame sow is at a higher risk of crushing piglets (Hill, 1990). The number of pigs born alive is lower in lame sows than in sound ones (Anil et al., 2009).

The prevention of problems is better than their cure, and it is ethically more acceptable. Management factors have a major influence on the incidence of lameness at the farm level (Heinonen et al., 2006). The risk of abnormal gait has been reported to increase on slatted floors compared with solid floors (Heinonen et al., 2006; Kilbride et al., 2009). The influence of floor type

on foot lesions and traumatic disorders is probably greater than on osteocondrosis (Brennan and Aherne, 1987). However, although the risk of lameness can be reduced by good management and preventive actions, it cannot be totally eliminated. Early intervention and medication of sick animals is important, both for medical and animal welfare reasons. NSAIDs are indicated in the treatment of lameness, not just for pain alleviation, but also for their anti-inflammatory actions. Meloxicam alleviated pain caused by non-infectious locomotor disorder and improved feed intake in lame pigs (Friton et al., 2003). It was administered as a single dose 0.4 mg/kg im and treatment was optionally repeated on the next day. Ketoprofen, particularly S-ketoprofen, has been reported to reduce interleukin-1 $\beta$ -induced cartilage destruction *in vitro* and thus to show activity against cartilaginous degradation (Panico et al., 2005).

## 6. AIMS OF THE STUDY

The aim of this thesis research was to investigate the enantiospecific plasma concentrations and efficacy of racemic ketoprofen after oral administration in pigs. The specific aims of the study were as follows:

1. To determine the plasma concentrations of S- and R-ketoprofen and relative bioavailability of both enantiomers after oral and intramuscular routes of administration of racemic ketoprofen in pigs;
2. To assess the bioequivalence of racemic ketoprofen after oral and intramuscular administration;
3. To determine the effective oral dose of racemic ketoprofen for treating induced endotoxemia in pigs;
4. To examine the efficacy and compare two doses of orally administered racemic ketoprofen in the treatment of clinical lameness caused by spontaneous non-infectious musculoskeletal disorders in sows and gilts.

## 7. MATERIALS AND METHODS

### 7.1 Study design

Studies I and II were performed as randomized crossover studies, each pig receiving ketoprofen either four times (study I) or two times (study II). The washout period was a minimum of six days between the treatments.

Study III was a blinded, placebo-controlled and randomized experimental trial.

Study IV was conducted as a randomized, double-blinded, placebo-controlled clinical field trial in ten privately owned farms in southern Finland.

All the studies were in compliance with current national ethical regulations.

### 7.2 Animals and clinical examinations

#### 7.2.1. *Animals*

Eight crossbred pigs, mean  $\pm$  SD weight  $51.8 \pm 8.4$  kg at the beginning of the study,  $77.9 \pm 9.9$  kg at the end of the study, were selected for study I. In study II, eleven crossbred pigs, 5 females and 6 barrows, were used. They were 9–13 weeks old at the beginning and their mean body weights  $\pm$  SD were  $36.5 \pm 2.8$  kg at the beginning of the trial and  $42.6 \pm 3.2$  kg at the end. For study III, 40 crossbred pigs approximately 10 weeks of age and weighting 21–35 kg were purchased.

In study IV, a total of 1955 sows and gilts (30–168 animals per herd) were included in the first stage of the study. In the small herds, numbering less than 500 sows, all eligible loose-housed sows and gilts were examined for signs of lameness. In units with more than 500 sows, the owner presented animals eligible for inclusion according to the instructions of the investigators. In these large herds, the maximum number of animals that could be inspected within one day (average of 104 sows) limited the number of animals included in the study. Lactating sows and sows pregnant for  $> 100$  days were excluded. A total of 282 animals with abnormal movements were selected for individual examination, and 162 of these were assessed to have a lameness score  $\geq 2$ . Fourteen animals were excluded after clinical examination for one or more of the following reasons: fractures, infected wounds on the legs, body temperature  $> 39.5$  °C or any other concurrent disease. In addition, seven animals were excluded due to protocol violation. A total of 141 animals, 114 sows and 27 gilts, were included in the final analysis. The number of animals varied between the study farms from 5 to 31. One healthy control animal was selected for each lame study animal. The

control animals were examined and sampled in the same manner as the lame animals, but they received no treatment.

The animals included in the studies had not received NSAIDs, systemic antibiotics or systemic glucocorticosteroids within 14 days before the start of the trials.

### 7.2.2. *Housing and management*

In the experimental studies (I, II and III), pigs were housed individually in pens (studies I and II) or in groups of ten, with the pigs of each phase in the same pen (study III). They were fed twice daily with a standard commercial feed with no antimicrobials. Water was offered *ad libitum*. In the field study (IV), the animals remained at the farm under the care and supervision of their owners during the study. Feeding and other husbandry practices remained unchanged during the trial.

### 7.2.3. *Clinical examinations*

The health status of the study animals was assessed on the basis of clinical examination, haematology and blood chemistry prior to the study procedures and before each drug administration in studies I and II.

In study III, the pigs were clinically observed by the same person throughout the study. The clinical examinations were performed on days -3, -2, -1, 0 (just before injection of endotoxin) and at 0.5, 1, 2, 4, 6, 8, 10 and 24 hours after the challenge. The general behaviour, respiratory rate and locomotion were scored (Table 3). The sum of these scores was calculated to form a total clinical score. Rectal temperature was measured using controlled thermometers.

In the first stage of study IV, the investigators evaluated the movement of pigs in groups for 2–5 min in their own pens. Some animals, whose movements could not be adequately observed in the pen, were examined on the gangway. All animals with abnormal movements were selected for individual examination. Two investigators independently assessed their lameness using a five-grade scoring table (Table 4) while the animals walked on a hard, solid floor for at least 10 m. Animals with a lameness score  $\geq 2$  assessed as identical by both investigators independently were included in the study. Secondly, each study animal underwent a thorough clinical examination. The lame leg was inspected and palpated. Rectal temperature was measured with a digital thermometer. The weight (kg) of the animals was calculated using the following formula:  $\text{circumference}^2 \text{ (cm)} \times \text{length (cm)} / 13\,781$  (Savage, 2004). The parity number was recorded from the farm data. On day 5, the last medication day, the study animals were re-examined a second time in the same way as described above.

Table 3. The scoring system used to assess the general behaviour, respiratory rate and locomotion in pigs challenged with *E. coli* endotoxin.

Score	General behaviour	Respiratory rate	Locomotion
0	Normal	Normal	Normal
1	Slightly depressed and/or reluctance to move	Increased	Slight incoordination
2	Highly depressed	Laboured	Marked ataxia
3	Comatose	Dead	Dead
4	Dead	-	-

Table 4. Lameness scoring system used to assess lameness in sows and gilts.

Score	Lameness	Clinical signs
0	None	No lameness
1	Minimal	Stiff, ataxic or swaying gait, shortened stride
2	Slight	Limp visible, but animal unconcerned and exercises normally
3	Moderate	Obvious limp present all the time (with head bobbing), animal having some difficulty with exercise, moderate kyphotic posture
4	Severe	Animal barely weight bearing/not weight bearing, severely lame but able to move, severe kyphotic posture

## 7.3 Treatments

### 7.3.1. *Racemic ketoprofen, dose rates and administration routes*

Racemic ketoprofen was administered via either the parenteral or per oral route (Table 5). All treatments in studies I to III were administered as a single dose, whereas the duration of the medication in study IV was five consecutive days.

Table 5. Racemic ketoprofen products used, routes of administration and dose rates.

Study	Drug	Route of administration	Dose (mg/kg)
I	Romefen <sup>1</sup>	iv, im	3
I	Dolovet <sup>2</sup>	po	3 & 6
II	Romefen	im	3
II	Ketovet <sup>3</sup>	po	4
III	Ketovet	po	0.5, 1, 2 & 4
IV	Ketovet	po	2 & 4

<sup>1</sup>Merial, Lyon, France. <sup>2</sup>Vetcare, Salo, Finland. <sup>3</sup>Galena, Kuopio, Finland.

The ear vein of the other ear from that used in blood sampling was used for iv administration. The injection site for im injection was the muscle on the right side of the neck. The ketoprofen powder was mixed with 40 mL of tap water and administered via a stomach tube for the oral route in studies I and II. The tube was flushed with 50 mL water before removal. In study III, ketoprofen powder was mixed with tap water at various concentrations so as to administer dose rates 0.5 mg/kg, 1 mg/kg, 2 mg/kg and 4 mg/kg under the same volume of 1 mL/kg. The control group received 1 mL/kg of tap water. Prior to administration, the vials containing the formulations were coded by a technician who was not involved in the experimental phase of the study. The test solutions were administered orally with a 20 mL syringe that was placed into the mouth of the pigs. No notable spilling was observed. Two to four mg/kg are referred to as high-dose groups and 0.5–1 mg/kg as low-dose groups.

In the field study (study IV), Ketovet oral powder was used as the test product for the dose of 4 mg/kg. The 50:50 mixtures of Ketovet oral powder and placebo were used for the 2 mg/kg dose. The placebo contained 14 g maltodextrine and 1 g of carmellose sodium, both of which are excipients of Ketovet oral powder. The University Pharmacy of Helsinki manufactured the 2 mg/kg mixture and placebo and re-packed and labelled all sachets. The visual appearance of the powder and the sachets was identical. The sachets were labelled in an identical manner and numbered with consecutive numbers. All pre-numbered sachets were divided randomly into 4 mg/kg and 2 mg/kg of ketoprofen, and placebo groups within blocks of six successive numbers. On each farm, a new block was used in order to ensure equal allocation of all the test products within the farms. On the basis of each animal's body weight, the correct daily doses were prepared for 5 days and packed into an unused plastic bottle with a cover. Before administration, 10 mL of tap water was added to the bottle, the mixture was shaken well and drawn into a 20 mL syringe. The treatment was administered via the oral route by placing the syringe deeply into the mouth of the animal to minimize spilling.



### 7.3.2. Allocation to the treatment groups

The pigs received the treatments in a random order in studies I and II. In study III the pigs were allocated to five groups of eight pigs based on weight and in such a way that the average weights were similar between the groups. The five groups were then randomly allocated to treatment groups A to E. For practical reasons, the study was conducted in four identical phases performed on consecutive days, each phase including two pigs from each group.

In the field study (study IV) the sows and gilts included in the study were allocated at random to one of the three treatment groups: placebo, ketoprofen 2 mg/kg or ketoprofen 4 mg/kg. Once an animal was included in the study, it was given the next available study number from the medication block used on that farm.

### 7.3.3. Endotoxin challenge

The pigs were challenged with *E. coli* endotoxin in study III. A solution of 20 µg/mL endotoxin *E. coli* lipopolysaccharide O55:B5 (Sigma) in physiological saline was prepared just before the challenge. The solution was injected intravenously (superficial brachial vein) at a dose rate 4 µg/kg body weight one hour before ketoprofen treatment.

## 7.4 Blood sampling

Heparinised tubes were used in studies I, II and III, and serum and EDTA tubes in study IV.

A vinyl tube (inner diameter 1.0 mm; outer diameter 1.5 mm, Sterihealth®, Australia) for venous blood sampling was inserted nonsurgically into the ear vein (studies I and II) or cephalic vein (study II) before the first sampling. Each pig was placed on its back and a soft rope snare was placed around the maxilla to provide restraint during placement of the tubing. The placement area was cleaned and disinfected. A 13-gauge catheter (Intraflon2, Vygon, Ecoen, France) was inserted into the auricular vein or cephalic vein, and approximately 50 cm of vinyl tube was threaded through the catheter into the vein. The 13-gauge catheter was removed, and a blunted, 18-gauge needle hub was inserted into the end of the vinyl tubing. A stopper was then inserted into the needle hub to prevent backflow. The vinyl tubing was filled with heparinised saline (0.9% NaCl) solution (5 U/mL). To prevent contamination with saline, the first 2 mL drawn up was discarded and the sample was taken with another unused 10 mL syringe. Blood samples were collected before each treatment, and then 2, 5, 10, 15, 30 and 45 minutes and 1, 2, 3, 4, 6, 8, 12, 24 and 48 hours after iv administration of ketoprofen, and 15, 30 and 45 minutes and 1, 1.25, 1.5, 2, 3, 4, 6, 8, 12, 24 and 48 hours after po and im ketoprofen administration in study I. In study II, blood samples were collected before each treatment, and then 30 and 45 minutes and 1, 1.25, 1.5, 1.75, 2, 4, 6, 8, 10, 12 and 24 hours after po and im administration of ketoprofen.

In study III the blood samples were collected from the *vena cava* before and 0.5, 1, 2, 4, 8, 10 and 24 hours after the endotoxin challenge. In the field study (IV), the samples were taken from the saphenous or tail vein on days 0 and 5.

## 7.5 Laboratory analysis

The serum (study IV) and plasma (studies I, II and III) samples were centrifuged and immediately frozen and stored at -18 °C or lower until analysed.

### 7.5.1. Total ketoprofen plasma concentration analysis

In study I, total plasma concentrations of racemic ketoprofen were determined by HPLC analysis according to the method described by Owen et al. (1987) with slight modifications. Determinations were conducted on 4 samples in parallel. The HPLC system was equipped with a piston pump Waters 501 (Waters Corp, Milford, MA, USA), Waters 717 autosampler (Waters Corp, Milford, MA, USA), Waters 486 tunable absorbance UV detector (Waters Corp, Milford, MA, USA) and Millennium 32 chromatography manager workstation (Waters Corp, Milford, MA, USA). Sample separation was conducted on a 4.6 X 150 mm column packed with 5 µm of reversed-phase silica SunFire C18 (Waters Corp, Milford, MA, USA). The flow rate of the isocratic mobile phase, which consisted of acetonitrile and 0.03% phosphoric acid (1:1), was 1.5 mL/min. The analytic wavelength was 254 nm. The method was validated as recommended for bioanalytic assays (Shah et al., 2000) by analysing 6 parallel plasma samples spiked with ketoprofen. The standard curve was found to be linear ( $r^2 > 0.998$ ) for the concentration range of 0.36 to 50 mg/L. The limit of quantification was 0.36 mg/L.

In study III the assay method was a modification of that previously described by Upton (1980). The procedure involved the liquid-liquid extraction of ketoprofen from plasma and reversed-phase chromatography with UV detection. The chromatographic system consisted of an Agilent 1100 HPLC with an Agilent ChemStation data system. The mobile phase was a mixture of acetonitrile and 0.05 M potassium dihydrogen phosphate, pH 4.4–4.6 (65:35, v/v). A mixture of 0.5 mL of plasma, an internal standard (ibuprofen, 500 ng) and 250 µL of 1 M phosphate buffer, pH 2, was extracted with 4 mL of diethyl ether. After centrifugation, the organic layer was separated and evaporated to dryness at 40 °C under a stream of nitrogen. The dried residue was reconstituted with 100 µL of the mobile phase and 50 µL was injected onto the HPLC column (Phenomenex Luna C18 (2), 250 x 4.6 mm, 5 µm). The effluent was monitored at 275 nm (ketoprofen) and 222 nm (ibuprofen) with a flow rate of 1.0 mL/min. Ketoprofen concentrations were quantified using linear regression of peak height ratios (ketoprofen/internal standard) vs. concentration. The calibration curve was linear from 0.1 to 7.5 µg/mL, and the lower limit of quantification was 0.1 µg/mL.

### 7.5.2. *Enantiospecific ketoprofen plasma concentration analysis*

Plasma concentrations of ketoprofen enantiomers were separated on a chiral column and detected by LC-MS/MS analysis. Organic solvents were of HPLC grade and other chemicals of analytical purity. Racemic ketoprofen used for standard curves and spiked samples was obtained from USP U.S. Pharmacopeia (Rockville, MD, USA) and racemic ketoprofen-d4 from Qmx Laboratories (Thaxted Essex, UK). Optically active S-ketoprofen was purchased from Aldrich (St Louis, MO, USA) and was used to identify the elution order of ketoprofen enantiomers. Appropriate standard solutions were prepared by dilution with methanol. For ketoprofen analysis, plasma samples were centrifuged (4000 rpm, 5 min) and a portion of a sample (0.5 ml) was diluted with ammonia (4%, 0.5 ml), subsequently followed by solid phase extraction (Oasis MAX, 1cc, 30 mg, Waters). Before sample loading, the cartridge was conditioned with methanol (1 ml) and water (1 ml). The cartridge was washed with ammonia (5%) in water (1 ml), methanol (1 ml) and formic acid (2%) in methanol/water (45/55, v/v, 1 ml). The analytes were eluted with formic acid (2%) in methanol/water (90/10, v/v, 1 ml). The solvent was evaporated to dryness under a stream of nitrogen at 45 °C and redissolved in the mobile phase (250 µl). An aliquot of a sample (10 µl) was injected into the LC-MS/MS.

Ketoprofen enantiomers were separated on a chiral column (Chirobiotic R, 15 cm x 2.1 mm, 5 µm, Supelco, Bellefonte, USA) protected with a guard column (C 18, 4x 2.0 mm, Phenomenex) using ammonium acetate buffer (20 mM, pH 5.6 adjusted with formic acid) in 30% methanol. The flow rate was 0.2 ml/min and the column temperature was set at 18 °C.

The LC-MS/MS instrumentation consisted of a Waters Alliance 2695 Separations Module (Waters, Milford, MA, USA) and a MicroMass Quattro Micro triple quadrupole tandem mass spectrometer (MicroMass Ltd, Manchester, UK) operated in the negative ion mode. The mass spectrometric parameters were: capillary voltage 3.0 kV; source temperature 120 °C; desolvation temperature 300 °C; N<sub>2</sub> cone gas flow 15 L/h and N<sub>2</sub> desolvation gas flow 500 L/h. Argon was used as a collision gas. The multiple reaction monitoring mode was used for monitoring ion transitions, which were m/z 253 for the deprotonated molecular ion [M-H]<sup>-</sup> and m/z 209 for the product ion. The transition m/z 257 to 213 was monitored for the racemic ketoprofen-d4, which was used as an internal standard. Peak integration and calibration were performed with QuanLynx software (MassLynx 4.0, Waters).

The matrix-matched calibration curve was found to be linear over the selected concentration range of 4–7000 ng/ml for each enantiomer by a weighted ( $1/x^2$ ) least-squares linear regression model. Method validation was performed in the lower concentration range of 4–60 ng/ml. Recovery and precision (repeatability and within-laboratory reproducibility) were measured by spiking six blank plasma samples at levels of 5, 15 and 50 ng/ml for both enantiomers on three different days (N = 18 for all concentrations). Recoveries varied between 94–109% for S-ketoprofen and 91–96% for R-ketoprofen. The coefficients of variation of repeatability (CV<sub>r</sub> %) were less than 8.7% for S-ketoprofen and less than 9.8% for R-ketoprofen, while the coefficients of variation of within-laboratory reproducibility (CV<sub>wIR</sub> %) were less than 11.2% and 10.2%,

respectively. The limit of detection was 2 ng/ml in plasma for both enantiomers evaluated from the lowest spiking level (5 ng/ml), which is considered as the limit of quantification of the method.

### 7.5.3. Analyses of thromboxane B<sub>2</sub> and haptoglobin plasma concentrations, and haematological parameters

Prior to immunoassay, TXB<sub>2</sub> extraction was performed in order to improve the quality of dosage by eliminating potential interfering compounds contained in plasmas. The extraction was performed by using the plateprep 96-well vacuum manifold starter kit (Sigma, France). TXB<sub>2</sub> was determined by an ELISA technique kit (Thromboxane B<sub>2</sub> Enzyme Immunoassay, Assay Designs, USA) at the CRIS Pharma Laboratory (Saint Malo, France). Each sample was tested in triplicate, but standards were assayed in duplicate. The absorbance was read at 405 nm with a Multiskan EX apparatus (Thermo Labsystems, France), the reference wavelength being 570 nm. The TXB<sub>2</sub> concentration was expressed in pg/ml.

Haptoglobin was measured by using the haemoglobin-binding assay for bovine (Makimura and Suzuki, 1982), with slight modifications in which tetramethylbenzidine (0.06 mg/ml) was used as a substrate (Alsemgeest et al., 1994) and the assay was adapted for microtitration plate use. Optical densities of the wells were read at 450 nm using a spectrophotometer (Multiskan MS, Labsystems Oy). The assay was calibrated using a reference porcine acute phase serum sample provided by the European Commission Directorate General Research Concerted Action Group (project number QLK5-CT-1999-0153). Haemolysis of the samples was estimated visually using a 3-grade scoring.

The haemoglobin concentration, packed cell volume, leukocyte and erythrocyte counts (study IV) were analysed using a Vet ABC Animal Blood Counter (ABX Diagnostics).

## 7.6 Pharmacokinetic parameters

Pharmacokinetic parameters were calculated by using Kinetica software (Thermo Electron Corp, Waltham, USA). The non-compartment analysis was applied for R- and S-ketoprofen plasma disposition curves. The AUC was calculated by use of the trapezoidal method. In each case, AUC<sub>0-12</sub> or AUC<sub>0-24</sub>, in studies I and II, respectively, was >80% of the calculated AUC<sub>0-∞</sub>. Values for C<sub>max</sub> and T<sub>max</sub> were directly determined from individual time vs. plasma concentration curves. The t<sub>1/2</sub> was calculated as 0.693/β, in which β is the elimination rate constant. The MRT was calculated as the area under the first-moment curve from time 0 to infinity divided by AUC<sub>0-∞</sub>. The MAT was calculated as MRT<sub>extravascular</sub> - MRT<sub>iv</sub>, where MRT<sub>extravascular</sub> is the MRT after po or im administration and MRT<sub>iv</sub> is the MRT after iv administration. Values for V<sub>d</sub> were calculated by use of the area method as V<sub>d</sub> = (dose/[AUC<sub>0-∞</sub> X β]), and CL was calculated as dose/AUC<sub>0-∞</sub>; values for V<sub>d</sub> and CL was standardized per kilogram of body weight. The relative bioavailability was determined for S and R-ketoprofen by calculating (AUC<sub>po</sub>/ AUC<sub>im</sub>) (D<sub>im</sub>/D<sub>po</sub>)\*100%. The im route was used as a reference.

## 7.7 Evaluation of efficacy in the treatment of lameness

The efficacy of the treatment was assessed according to the lameness score on day 5. A lameness score of 0 represented excellent efficacy and a lameness score of 1 represented good efficacy. If the lameness score decreased from 4 to 3, from 3 to 2 or from 4 to 2, the efficacy of the treatment was considered fair. No improvement or deterioration in the lameness score was considered as poor efficacy. Excellent and good efficacy scores were considered as a successful outcome for the treatment.

## 7.8 Statistical analysis

Pharmacokinetic parameters obtained for S- and R-ketoprofen within administration groups were compared by use of the paired Student's *t*-test (AUC,  $C_{max}$ ,  $T_{1/2}$ , MRT,  $F_{rel}$ ) or Wilcoxon matched-pairs rank test ( $T_{max}$ ). As ketoprofen pharmacokinetics is linear, the dose was normalized ( $AUC_{norm}=(D_{im}/D_{po}) \times AUC_{po}$ ) when AUCs were statistically compared between administration groups.

To assess bioequivalence, the 90% CI was calculated for  $AUC_{0-12}$  in study I, and for  $C_{max}$ , all of which were logarithmically transformed. The 90% CI should be within an acceptance interval of 0.80 to 1.25 (EMA, 2001).

Statistical analyses in study III were performed using commercial software (SAS System for Windows, version 9.3). Repeated measures analyses of covariance (RM ANCOVA) models were constructed for rectal temperatures and  $TXB_2$  values. The models included treatment group, time, baseline rectal temperature/ $TXB_2$  value, sex and an interaction term between the treatment group and time as fixed effects, and the pig as the only random effect. Compound symmetry was used as the covariance structure. Based on the parameters of the fitted models, comparisons between treatment groups over time periods and at 1 hour were conducted together with within treatment group comparisons between time points (comparisons against baseline). Dunnett's test was used for the pairwise comparisons against the baseline.

In addition, a similar model where the four ketoprofen groups were put together was constructed for  $TXB_2$  to achieve a comparison between pigs that received the drug and pigs treated with placebo. The difference between the two groups was calculated over time from the 2-hour time point forward, as the drug was given at 1 hour. A subject-specific cumulative logit model was constructed for the total clinical score. The model included the main effects for the treatment group and time as fixed effects and the pig as the random effect. No other terms could be inserted into the model as fixed effects because there were very few observations in the higher categories of the total clinical score. A model where ketoprofen groups were put together was also constructed for total clinical score similarly, as with  $TXB_2$ . In this model, an interaction term between treatment group and time and sex was inserted into the model as a fixed effect. The differences between groups in the total clinical score were quantified with odds ratios and their 95% confidence intervals. The median and range of total ketoprofen plasma concentrations one hour after ketoprofen treatment

were calculated. Correlations between the plasma concentration of TXB<sub>2</sub> at 60 min post-administration and the rectal temperature and clinical signs were compared with Spearman's rho test.

In the field study (IV), statistical analyses were performed and tables produced using SAS software (SAS Institute, version 8.2). Blood parameters were analysed with Stata Intercooler version 10.0. The statistical unit was the sow. Treatment groups were compared using the Cochran-Mantel-Haenszel test with respect to the lameness score on day 0, parity number, change in lameness score from day 0 to day 5, treatment success in relation to the parity number and the efficacy of the treatment. Treatment success stratified by farm, parity and lameness score on day 0 was compared using a stratified CMH test. A logistic model was used to evaluate the interaction between slatted floor housing and the treatment group (ketoprofen groups were pooled), with treatment success as an outcome. Haemoglobin concentrations, PCV, erythrocyte and leukocyte counts, and haptoglobin concentrations in the three treatment groups and in the control animals were compared using ANOVA.

Descriptive data were presented as the mean values and SD or SEM of groups. For all tests,  $p < 0.05$  was considered significant.

## 8. RESULTS

### 8.1 Ketoprofen plasma concentrations

The peaks corresponding to racemic ketoprofen retention time (study I) were 4 min, and to the S- and R-ketoprofen (study II) 7.0 min and 8.4 min, respectively. Representative ion chromatograms of porcine plasma spiked with 30 µg/mL for racemic ketoprofen and 200 ng/mL for both enantiomers are presented in Figures 1 and 2, respectively.

The mean plasma concentration profiles of total ketoprofen following po, im and iv administration, and of S- and R-ketoprofen following po and im administration of racemic ketoprofen to healthy pigs (studies I and II) are illustrated in Figures 3 and 4, respectively. The plasma S-ketoprofen was above the LOQ for all sampling points after drug administration, except in one pig after the po route and three pigs after the im route at 24 hours post-administration. In most pigs (seven animals after po administration and nine after im administration), R-ketoprofen was above the LOQ for four hours or less. The remaining animals had quantifiable values for up to 24 hours. The second peak was evident in S-ketoprofen concentrations in plasma in most pigs, and it was more obvious after po administration than after im administration. It was not detected in R-ketoprofen (Figure 5).

In study III, the medians (range) of total ketoprofen plasma concentrations in endotoxin challenged pigs one hour after ketoprofen treatment were 0.1 µg/mL (<0.1–1.0), 0.19 µg/mL (<0.1–1.57), 0.83 µg/mL (<0.1–2.99) and 2.09 µg/mL (0.34–7.87) in the ketoprofen treatment groups 0.5 mg/kg, 1 mg/kg, 2 mg/kg and 4 mg/kg, respectively.

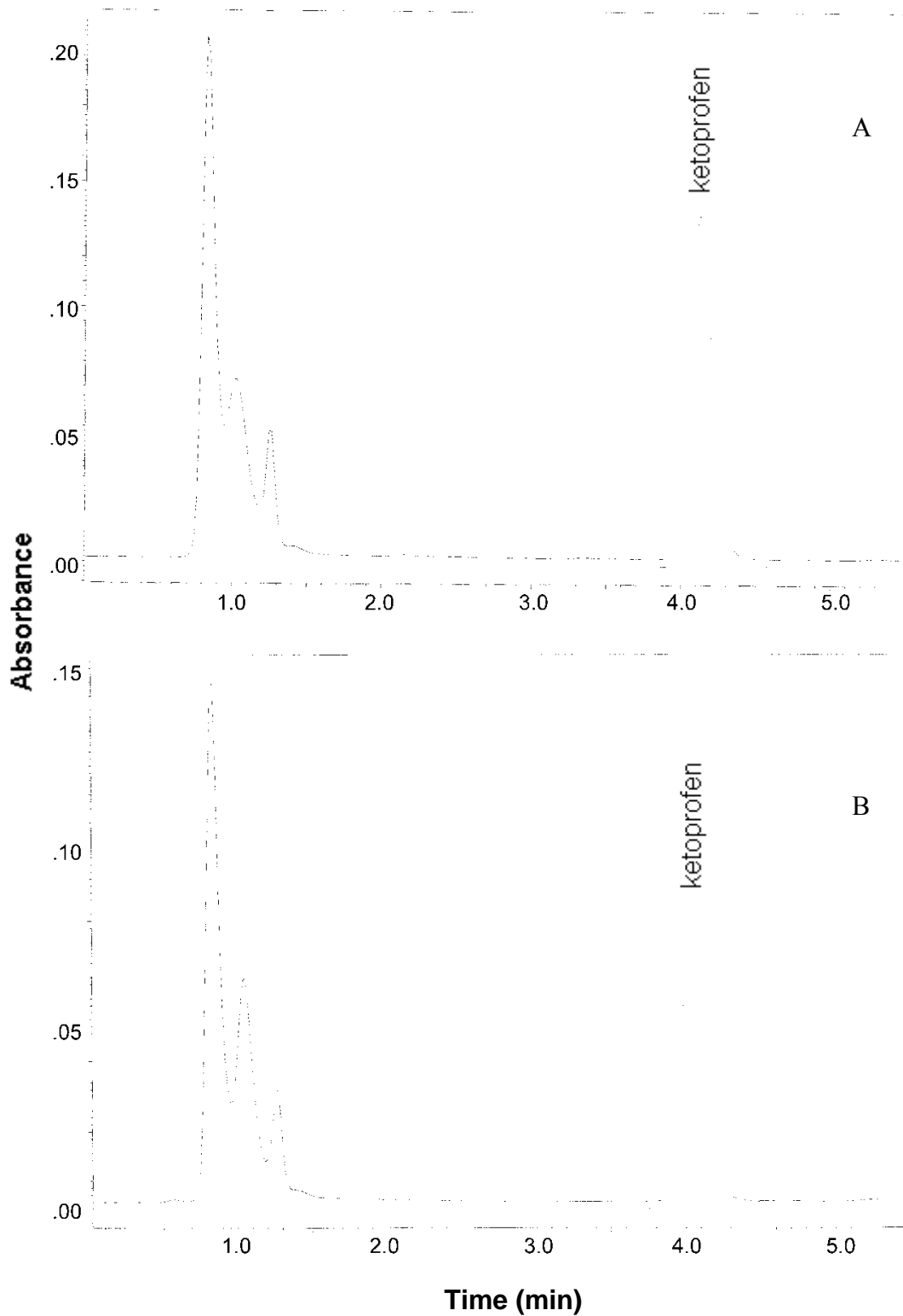


Figure 1. Representative chromatograms of porcine plasma spiked with racemic ketoprofen (30  $\mu\text{g/mL}$ ; A) and a plasma sample obtained from a pig 5 min after administration of racemic ketoprofen (3 mg/kg, iv; B). The total ketoprofen concentration in the treated pig was 23.8  $\mu\text{g/mL}$ .



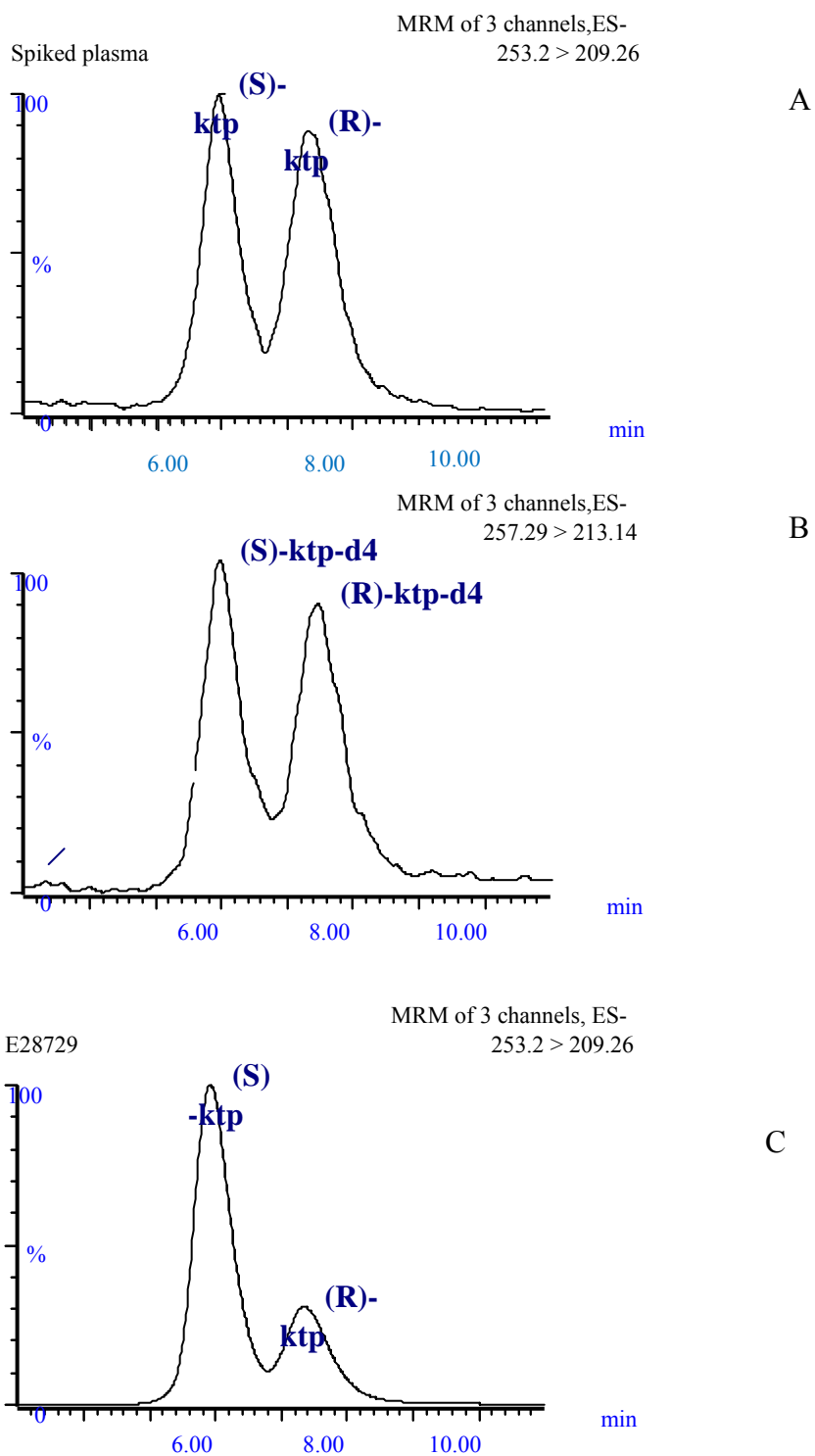


Figure 2. LC-MS/MS chromatograms of (A) porcine plasma spiked with racemic ketoprofen (100 ng/mL for each enantiomer) and (B) the corresponding internal standard (50 ng/mL for each enantiomer), and (C) a plasma sample obtained from a pig at 45 minutes after oral administration of racemic ketoprofen (4 mg/kg).

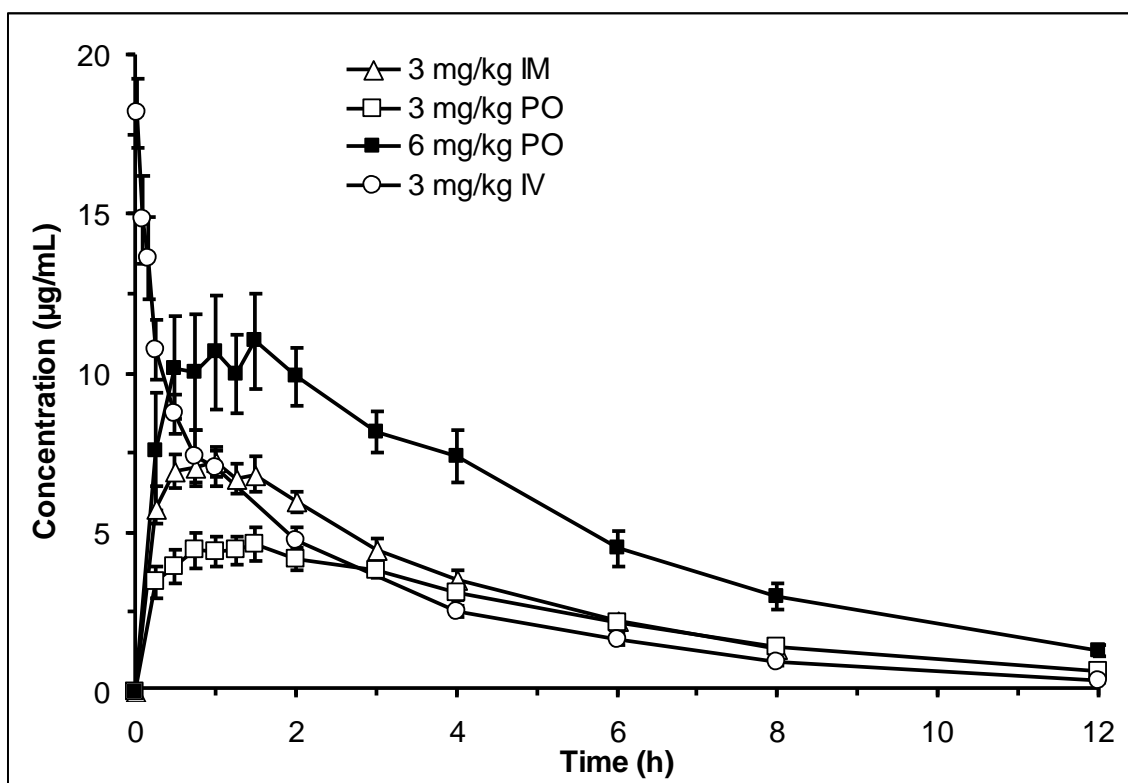


Figure 3. Mean  $\pm$  SEM total plasma ketoprofen concentrations in 8 pigs after administration of a single dose of racemic ketoprofen by various routes of administration. Ketoprofen was administered at time 0.

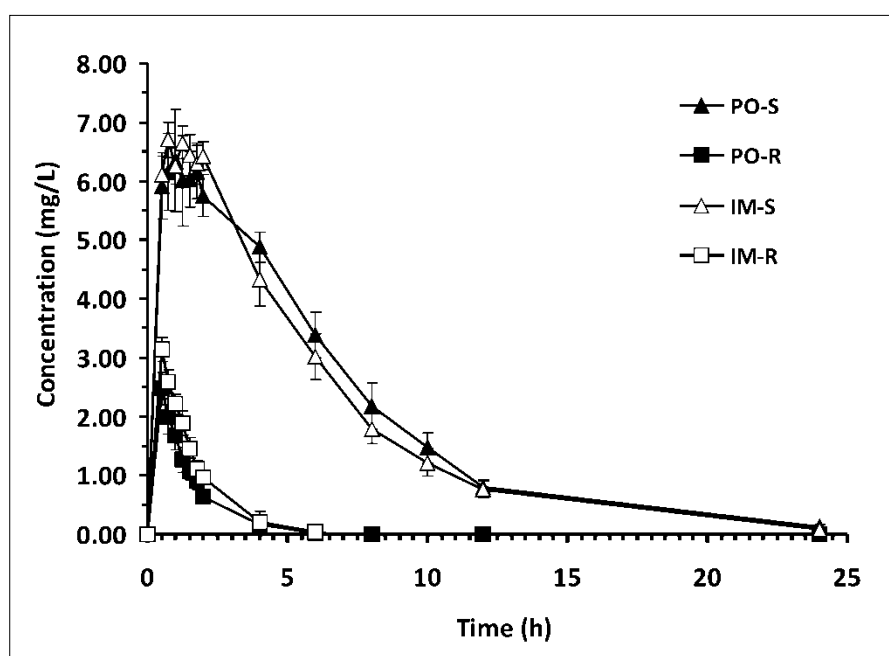


Figure 4. Mean  $\pm$  SEM plasma concentrations of S- and R-enantiomers in 11 pigs after administration of single doses of racemic ketoprofen, 3 mg/kg im and 4 mg/kg po. Ketoprofen was administered at time 0.

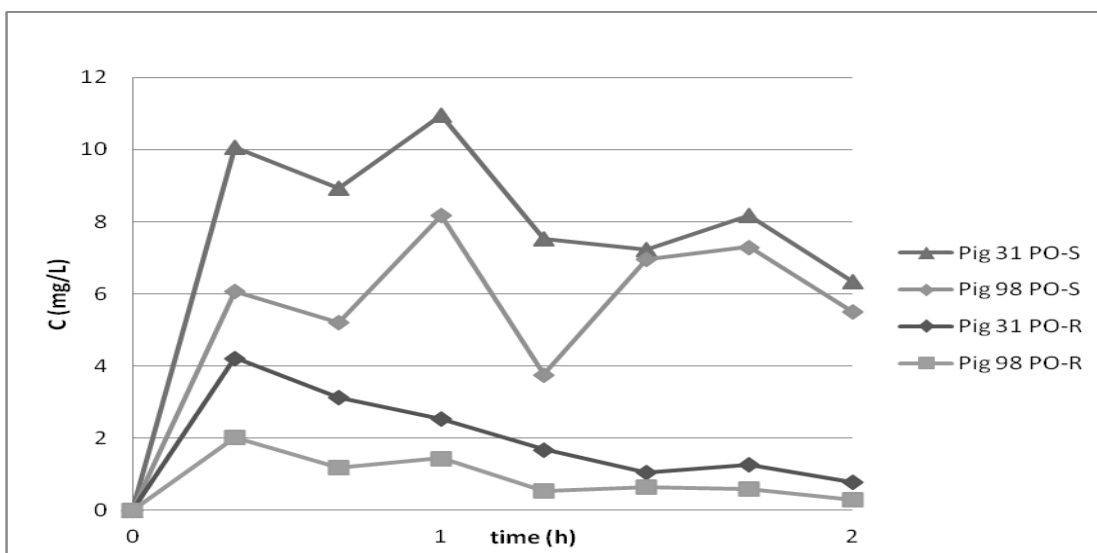


Figure 5. Individual plasma S- and R-ketoprofen concentration profiles of two pigs the first two hours after PO administration of racemic ketoprofen at dose rate of 4 mg/kg.

## 8.2 Pharmacokinetic parameters

Pharmacokinetic parameters for total plasma ketoprofen, and for plasma S- and R-ketoprofen are presented in Tables 6 and 7, respectively.

Bioavailability of total ketoprofen was almost complete after im and po administration. Mean  $\pm$  SD relatively bioavailability (po vs. im administration) of total ketoprofen in study II was  $89.2 \pm 3.1\%$ .  $F_{rel}$  (%) was  $83\% \pm 20\%$  and  $63\% \pm 23\%$  ( $p = 0.01$ ) for S-ketoprofen and R-ketoprofen, respectively.

Table 6. Pharmacokinetic parameters (mean  $\pm$  SD) of ketoprofen in pigs ( $n = 8$ ) after single doses of 3 mg/kg po, 6 mg/kg po, 3 mg/kg im and 3 mg/kg iv.

Parameter	3 mg/kg po	6 mg/kg po	3 mg/kg im	3 mg/kg iv
$T_{1/2}$ (h)	$3.52 \pm 0.90$	$3.22 \pm 0.43$	$2.95 \pm 0.21$	$2.66 \pm 0.50$
MRT (h)	$5.54 \pm 1.28$	$5.15 \pm 0.86$	$4.49 \pm 0.44$	$3.40 \pm 0.51$
MAT (h)	$2.15 \pm 1.01$	$1.75 \pm 1.11$	$1.09 \pm 0.35$	-
$T_{max}$ (h)	$1.25 \pm 0.90$	$1.19 \pm 0.46$	$1.06 \pm 0.48$	-
$C_{max}$ ( $\mu\text{g mL}^{-1}$ )	$5.09 \pm 1.41$	$12.03 \pm 4.81$	$7.62 \pm 1.22$	-
$AUC_{0-12}$ ( $\mu\text{g mL}^{-1} \text{ h}$ )	$28.3 \pm 6.7$	$63.4 \pm 17.6$	$34.6 \pm 6.5$	$32.4 \pm 7.6$
$AUC_{0-\infty}$ ( $\mu\text{g mL}^{-1} \text{ h}$ )	$32.04 \pm 9.20$	$69.51 \pm 19.32$	$37.14 \pm 6.99$	$33.86 \pm 8.45$
F (%)	$96.7 \pm 27.1$	$104.9 \pm 26.6$	$114.6 \pm 29.9$	-
CL ( $\text{L h}^{-1} \text{ kg}^{-1}$ )	-	-	-	$0.094 \pm 0.024$
$V_d$ (L/kg)	-	-	-	$0.35 \pm 0.09$

- = Not determined.

Table 7. Mean values ( $\pm$  SD) of pharmacokinetic parameters for ketoprofen after po or im administration of a single dose to 11 crossbred pigs.

Parameter		Route of administration	
		po 4 mg/kg	im 3 mg/kg
$C_{\max}$ (mg/L)	S-Ketoprofen	7.42 $\pm$ 2.35*	7.32 $\pm$ 0.75*
	R-Ketoprofen	2.55 $\pm$ 0.99	3.23 $\pm$ 0.70
$T_{\max}$ (h)	S-Ketoprofen	1.91 $\pm$ 1.65*	1.27 $\pm$ 0.45*
	R-Ketoprofen	0.59 $\pm$ 0.23	0.65 $\pm$ 0.30
$AUC_{0-\infty}$ ([mg/L]) $\cdot$ h	S-Ketoprofen	47.04 $\pm$ 13.41*	44.09 $\pm$ 12.87*
	R-Ketoprofen	3.83 $\pm$ 1.23	5.16 $\pm$ 2.48
$T_{1/2}$ (h)	S-Ketoprofen	3.40 $\pm$ 0.91*	2.89 $\pm$ 0.85*
	R-Ketoprofen	1.1 $\pm$ 0.90	0.75 $\pm$ 0.48
MRT (h)	S-Ketoprofen	5.55 $\pm$ 1.45*	4.91 $\pm$ 1.21*
	R-Ketoprofen	1.73 $\pm$ 0.92	1.49 $\pm$ 0.67
$AUC_S/AUC_R$ ratio		12.8 $\pm$ 3.17**	9.2 $\pm$ 2.39**

\* Denotes a significant difference ( $p < 0.05$ ) between S-ketoprofen and R-ketoprofen when comparing within administration groups. \*\* Denotes a significant difference ( $p < 0.05$ ) between administration routes (po versus im).

### 8.3 Bioequivalence

Equivalence could not be detected for  $AUC_{0-12}$  values of the total ketoprofen plasma concentration between 3 mg/kg administered po and iv (90% CI, 0.75 to 1.02), 3 mg/kg administered im and iv (90% CI, 0.90 to 1.29), or 3 mg/kg administered po and im (90% CI, 0.66 to 0.99). Neither was equivalence detected for  $C_{\max}$  of the total ketoprofen plasma concentration between 3 mg/kg administered po and im (90% CI, 0.53 to 0.80).

### 8.4 Rectal temperatures and total clinical scores

Mean rectal temperatures and total clinical scores are presented in Figures 6 and 7, respectively. The sex had no effect. No differences between pigs were detected in the rectal temperature at 1 hour after endotoxin challenge before ketoprofen treatment ( $p > 0.05$ ). The rectal temperature and the total clinical scores were elevated for up to 4 hours after endotoxin exposure in the control group, up to 2 hours in two ketoprofen low-dose groups and at 1 hour in two high-dose groups, although the differences in the total clinical scores between the groups were minor. The dose rates 2 and 4 mg/kg reduced rectal temperatures compared to the control group ( $p = 0.007$  and  $p = 0.027$ , respectively). The overall treatment effect of the mean rectal temperatures was 0.35 °C and 0.29 °C lower in ketoprofen 2 and 4 mg/kg groups, respectively, than in the control group. Ketoprofen treatment significantly lowered the total clinical score compared to the placebo group, although when comparing separate dose rates with the control group no significant differences were detected.

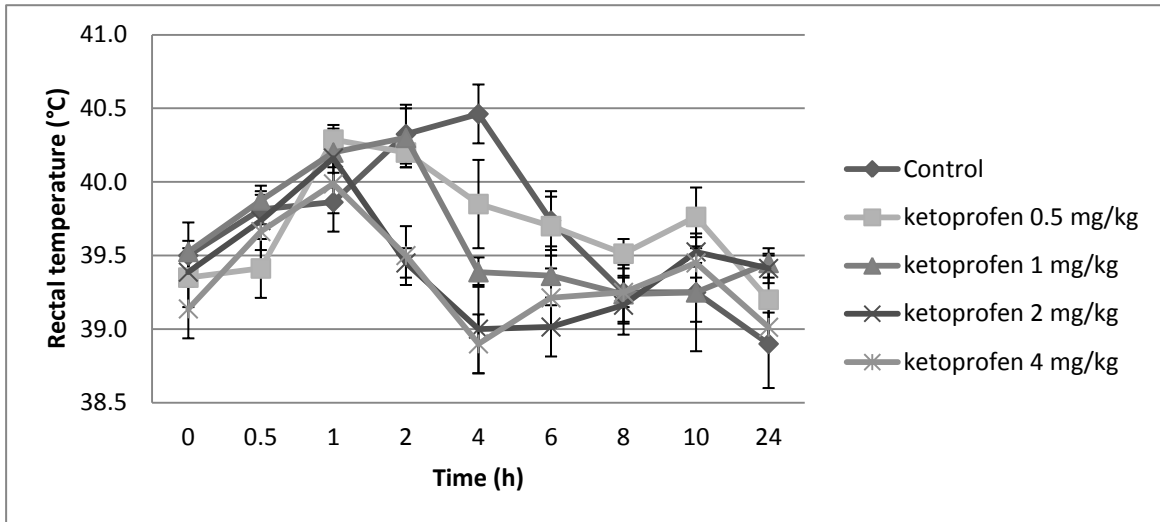


Figure 6. Effects of oral ketoprofen on the mean  $\pm$  SEM rectal temperatures in pigs after intravenous challenge with *E. coli* endotoxin. The treatments were administered one hour (T1h) after endotoxin challenge (T0h). (n = 40)

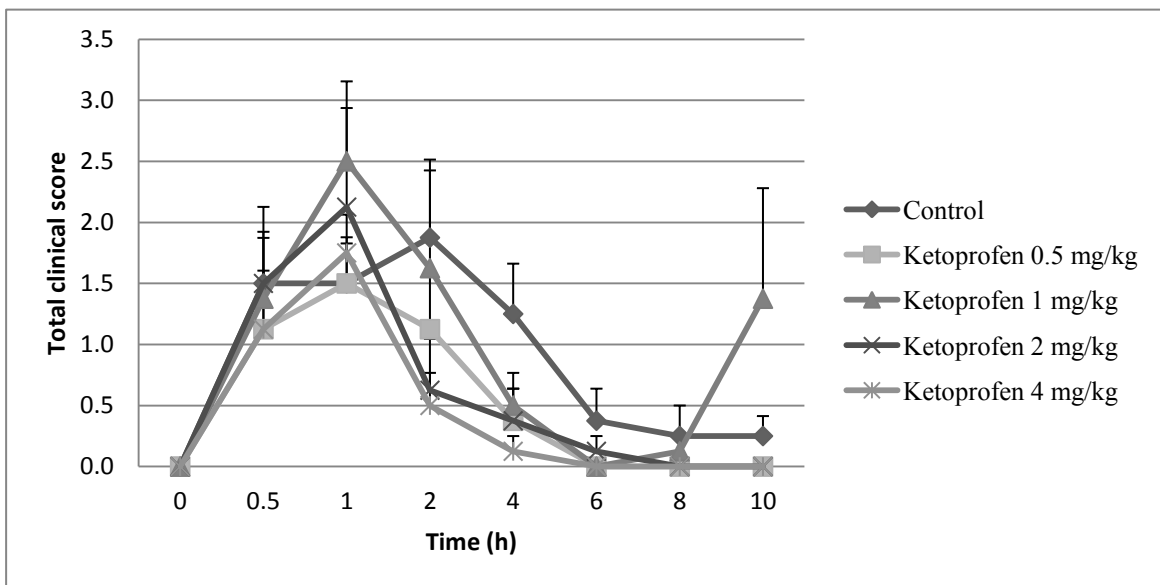


Figure 7. Effects of ketoprofen on the mean  $\pm$  SEM total clinical score in pigs after intravenous challenge with *E. coli* endotoxin. The treatments were administered one hour (T1h) after endotoxin challenge (T0h). (n = 40)

### 8.5 TXB<sub>2</sub> plasma concentrations and laboratory analysis

Mean plasma TXB<sub>2</sub> concentrations are presented in Figure 8. No significant differences between pigs were detected in the TXB<sub>2</sub> concentration at 1 hour after endotoxin challenge before ketoprofen treatment. The plasma TXB<sub>2</sub> concentration was elevated for up to 4 hours after the endotoxin challenge in the control group and up to 1 hour in the groups treated with ketoprofen ( $p < 0.01$ ).

Ketoprofen treatment reduced a TXB<sub>2</sub> concentration of 1012 pg/mL (95% CI, -1848 to -177) between 2 to 10 h post endotoxin challenge compared to the control group ( $p = 0.02$ ), although no significant differences were detected between separate dose rates. Plasma concentrations of TXB<sub>2</sub> at 1 hour correlated significantly with clinical signs (coefficient 0.57), but not with the rectal temperature (-0.24).

The haemoglobin and haptoglobin concentrations, packed cell volume, leukocyte and erythrocyte counts blood values of the animals in study IV were within the normal range or showed only minor changes both before and after ketoprofen medication (Friendship et al., 1984; Heinonen et al., 2006).

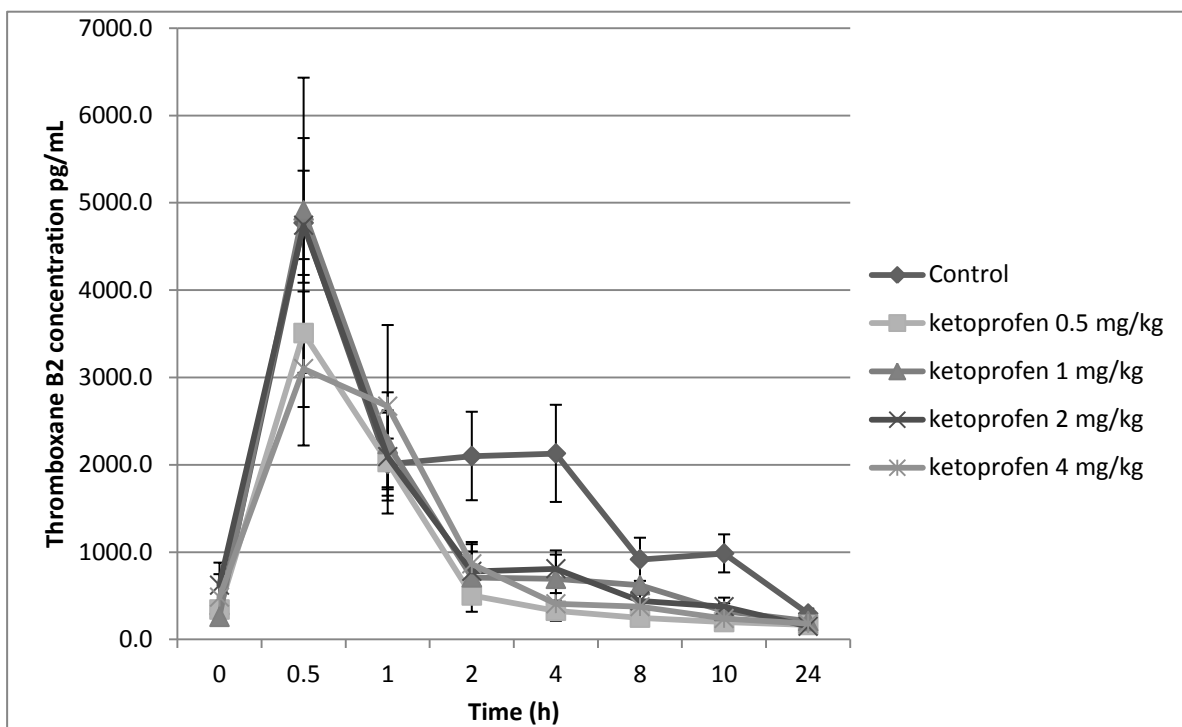


Figure 8. Effects of oral ketoprofen on the mean  $\pm$  SEM TXB<sub>2</sub> concentrations in pigs after intravenous challenge with *E. coli* endotoxin. The treatments were administered one hour (T1h) after endotoxin challenge (T0h). (n =40)

### 8.6 Efficacy in the treatment of lameness

The ketoprofen 4 mg/kg group comprised 46 pigs, the ketoprofen 2 mg/kg group 47 pigs, and the placebo group 48 pigs. Approximately 20% of each group comprised gilts, 50% sows with a parity of one to three, and 30% sows with a parity of four or more ( $p = 0.91$ , CMH test). The lameness scores of the animals in different treatment groups did not differ in the beginning (day 0) of the study (table 8). Mean changes of lameness scores from day 0 to day 5 appear in Table 9, and the efficacy of treatment in different groups appears in Table 10. The treatment was considered successful for 54.3% of the animals in the ketoprofen 4 mg/kg group, 53.2% of the animals in the ketoprofen 2 mg/kg group, and 20.8% of the animals in the placebo group. The difference between

both ketoprofen groups and the placebo group was significant ( $p = 0.001$ , CMH test stratified by farm), but the difference between the two ketoprofen groups was nonsignificant ( $p = 0.78$ , CMH test stratified by farm). The treatment successes of the three parity groups showed no differences ( $p = 0.21$ , CMH test stratified by farm).

Fully slatted floors had no direct impact on the success rate ( $p = 0.43$ ), but the interaction between the treatment and slatted floor was significant ( $p = 0.04$ , from the logistic regression model where the ketoprofen groups were combined). On both of the floor types, the ketoprofen groups had higher success rates than the placebo group, but the differences were more pronounced in farms with concrete or only partly slatted floors.

Table 8. Lameness scoring of 141 lame animals on day 0

Lameness score	Total	Ketoprofen 4 mg/kg n of animals	Ketoprofen 2 mg/kg n of animals	Placebo n of animals
	n (%) of animals			
2 Slight	81 (57%)	30	27	24
3 Moderate	58 (41%)	15	20	23
4 Severe	2 (1%)	1	0	1
Total	141	46	47	48

The lameness scores did not differ significantly between the three treatment groups on day 0 ( $p = 0.37$ ).

Table 9. Average lameness scores and their mean changes in 141 lame sows and gilts before (day 0) and after (day 5) per oral ketoprofen (two dose groups) or placebo treatment for 5 days

Treatment group	Animals <i>n</i>	Mean score on day 0 ( $\pm$ SD)	Mean score on day 5 ( $\pm$ SD)	Mean change ( $\pm$ SD)
Ketoprofen 4 mg/kg	46	2.37 (0.53) <sup>a</sup>	1.5 (0.98) <sup>a</sup>	0.87 (0.98) <sup>a</sup>
Ketoprofen 2 mg/kg	47	2.43 (0.50) <sup>a</sup>	1.38 (0.97) <sup>a</sup>	1.0 (1.0) <sup>a</sup>
Placebo	48	2.52 (0.55) <sup>a</sup>	2.08 (0.82) <sup>b</sup>	0.44 (0.82) <sup>b</sup>

<sup>a,b</sup> Groups with different superscripts within the column differ significantly ( $p < 0.05$ ).

Table 10. Efficacy of treatment of 141 lame sows or gilts in three treatment groups: ketoprofen 4 mg/kg, 2 mg/kg and placebo orally for 5 days

Efficacy	Ketoprofen 4 mg/kg, number (%) of animals	Ketoprofen 2 mg/kg, number (%) of animals	Placebo number (%) of animals
Excellent	7 (15%)	10 (21%)	2 (4%)
Good	18 (39%)	15 (32%)	8 (17%)
Fair	4 (9%)	8 (17%)	10 (21%)
Poor	17 (37%)	14 (30%)	28 (58%)

### 8.7 Adverse events

All pigs in the study II were euthanized after completing the study, and sent for necropsy. Signs of peptic ulcers were found in seven out of eleven animals. All the ulcers were in the glandular part of the stomach except in one pig, which had ulcers in the oesophageal area. None of them showed clinical signs or any abnormalities in blood values.

In study III, two pigs treated with 1 mg/kg ketoprofen died between 10 and 24 hours after the endotoxin challenge. Clinically, one of them had laboured breathing during the first two hours after the endotoxin challenge. Otherwise, both pigs were clinically normal when compared to other animals in the same group up to 10 hours after the challenge. At 10 hours, their general behaviour was depressed and they had marked ataxia. One of the pigs was hypothermic (37 °C), while the other had a rectal temperature within the reference range (38.8 °C). Their plasma TXB<sub>2</sub> concentrations remained high, despite ketoprofen treatment. Post-mortem examination of the hypothermic pig failed to reveal any macroscopic pathological signs, whereas fatty degeneration of the liver and hyperemia of the gastrointestinal tract were observed in the other dead pig. The latter could have been at least partially a post-mortem change. Plasma ketoprofen concentrations at one hour after its administration were below the limit of quantification and 0.23 µg/ml in the hypothermic and normothermic pig, respectively.

In the two other studies (studies I and IV), tolerance of ketoprofen was good, and no adverse events were observed.



## 9. DISCUSSION

Orally administered racemic ketoprofen was shown to be absorbed well and was efficacious in the treatment of endotoxemia and lameness in pigs. Clinical tolerance of ketoprofen treatment was good and it was easy to administer, even to loose-housed sows.

### 9.1 Pharmacokinetics of orally administered racemic ketoprofen

The absolute bioavailability of both ketoprofen enantiomers after im administration is suggested to be almost complete (Fosse et al., 2011a; Fosse et al., 2011b). After po administration, bioavailability is reported to be lower but similar, approximately 85%, for both enantiomers (Neirinckx et al., 2011a). The results of our studies are consistent with previous findings; the bioavailability of racemic ketoprofen was almost complete in pigs after po and im administration. The relative bioavailability of S-ketoprofen after oral administration was significantly higher than for R-ketoprofen. Since S-ketoprofen is generally regarded as the eutomer regarding cyclooxygenase inhibition, and the terminal half-life of R-ketoprofen is short, in clinical use the administration routes at dose rates used in this study could be considered equally effective.

S-ketoprofen was the predominant enantiomer in pig plasma after administration of the 50:50 racemic drug via both routes, as in most animal species previously studied. Our results agreed with other studies in pigs (Larsen et al., 1991; Fosse et al., 2011a; Neirinckx et al., 2011a). The pharmacokinetic parameters of both enantiomers after im administration were similar to those reported by Fosse (2011a) in young piglets with only minor differences, despite the pigs in our study being older than those used in their study.  $T_{max}$  and  $AUC_S/AUC_R$  ratio after oral administration were higher in our study than in the study reported by Neirinckx (2011a). The ketoprofen product used in that study was an oral solution, which could have been absorbed faster than the oral powder we used.

The higher relative bioavailability of S than R ketoprofen in our study suggests that some stereoselective absorption and/or first pass metabolism may have occurred. The physicochemical properties of the enantiomers are identical and the absorption of ketoprofen is not therefore considered stereoselective. Absorption has been regarded as mainly a passive process, but there is also some evidence suggesting that ketoprofen may have an active transport pathway across the intestinal wall (Foster and Jamali, 1988; Foster et al., 1988a; Foster et al., 1988b; Jamali and Brocks, 1990; Aberg et al., 1995; Choi et al., 2006). In rats, 84% of the administered dose of R-ketoprofen was inverted to S-ketoprofen in the gastrointestinal tract (Foster and Jamali, 1988), while an absence of pre-systemic inversion was reported in pigs (Neirinckx et al., 2011b). The inversion rate of R-ketoprofen to S-ketoprofen is equally high in rats and pigs (approximately 70%) (Aberg et al., 1995; Neirinckx et al., 2011b). Presystemic inversion in the gastrointestinal tract has

also been reported in rats after ibuprofen and fenoprofen administration, and was dependent on the absorption rate (Berry and Jamali, 1991; Sattari and Jamali, 1994). However, presystemic inversion of R-benaxoprofen to S-benaxoprofen detected in gut preparations was weak compared to inversion in the liver and kidney (Simmonds et al., 1980; Nakamura and Yamaguchi, 1987). The possible faster absorption rate of the oral solution used by Neirinckx (2011a) may have been partially responsible for the absence of possible pre-systemic inversion found in their study. In our study, the slower absorption rate from the gastro-intestinal tract might have contributed the pre-systemic inversion. Ketoprofen solution has reported to reach the maximum plasma concentration earlier than the tablet formulation (Stiegler et al., 1995). The racemic ketoprofen used in our studies was an oral powder, which was insoluble in water. In the present study, the difference in relative bioavailability between enantiomers was smaller, although significant, than that reported in rats after po and ip administration (Foster and Jamali, 1988). After ip dosing, the AUCs of both enantiomers were similar to those after iv dosing, which would suggest that presystemic inversion occurs in the gastrointestinal tract. In our study, fluctuation evident in total plasma ketoprofen concentrations was also observed in S-ketoprofen plasma concentrations after po and im administration. There was individual variation in the sharpness of the fluctuation in plasma concentrations of S-ketoprofen, and the peak was more evident after po administration of ketoprofen than after im administration. In most of the pigs, it was clearly evident within two hours after per oral ketoprofen administration. It may have been caused by enterohepatic recycling. The phenomenon was not detectable for R-ketoprofen. Stereoselective enterohepatic circulation of ketoprofen exists at least in rats (Yasui et al., 1996). Yasui et al. (1996) suggested that the glucuronide of S-ketoprofen is hydrolyzed more slowly than the glucuronide of R-ketoprofen in the intestine. This would lead to a longer mean transit time of S-ketoprofen from the bile duct via the intestinal tract and into the systemic circulation, and stereoselective enterohepatic recycling may therefore occur. However, the high degree of chiral inversion from S- to R-ketoprofen could also explain some fluctuation in the plasma S-ketoprofen concentration, especially as it was more evident after po than im administration.

Since the difference in AUC values between enantiomers was significant for po and im administration routes, the most probable reason for the lower AUC of R-ketoprofen was the higher clearance compared to S-ketoprofen. The enantioselective differences in clearance may be attributable to differences in distribution, chiral inversion, hepatic metabolism, renal excretion, or to a combination of these factors. The volume of distribution for ketoprofen is low, 0.35 in our study, probably due to high protein binding. Enantioselectivity in the binding of ketoprofen to plasma proteins has been reported in humans and camels (Dubois et al., 1993, Al Katheeri et al., 2000), although contradictory results have also been reported in humans (Lagrange et al., 2000). There have been no reports of possible enantioselective protein binding in pigs.

In our study the terminal half-life and MRT of R-ketoprofen were approximately one-third of and  $T_{max}$  a half of that for S-ketoprofen after po and im administration. The inversion rate of R-ketoprofen to S-ketoprofen is 70% in pigs (Neirinckx et al., 2011b), and pigs could be regarded as “extensive inverters”, as the inversion rate is above 50% (Aberg et al., 1995). The inversion process generally takes place in various organs (liver, intestine, kidney, and lung) and tissues (fatty and

muscle tissues), with the liver considered to be the most important organ (Cox et al., 1985; Mehvar and Jamali, 1988; Hall et al., 1992). Ketoprofen is metabolized in the liver and converted into a carbonyl-reduced derivative, 2-(phenyl 3- $\alpha$ -hydroxybenzoyl) propionic acid in swine (EMEA, 1996). In rats, stereoselectivity has been reported for the biliary excretion process (Menzel et al., 1993). The significance of stereoselectivity in reductive metabolism is difficult to assess, as inversion occurs in most species, and is rapid for ketoprofen (McEwen et al., 1998). Glucuronidation appears to be an important metabolic pathway for ketoprofen in pigs (EMEA, 1996). Stereoselectivity of glucuronidation has been reported, but it is species and compound dependent (Caldwell et al., 1988). The possible stereoselectivity of ketoprofen glucuronidation in pigs has not been studied, but it could to some extent explain the more rapid elimination of R-ketoprofen than S-ketoprofen.

## 9.2 Ketoprofen plasma concentrations

Ketoprofen concentrations persisted longer in plasma after oral administration of racemic ketoprofen than after intramuscular or intravenous administration at the same dose level.

Fosse et al. (2011b) reported an  $IC_{50}$  for S-ketoprofen of 26.7  $\mu\text{g/mL}$  and an  $IC_{50}$  for R-ketoprofen of 1.6  $\mu\text{g/mL}$  from mechanical nociceptive threshold testing in the kaolin-induced inflammation model in neonatal piglets. There have been no other reports on either the total or the enantiospecific therapeutic target concentration of ketoprofen in plasma for pigs. A total ketoprofen plasma concentration of 0.4–6  $\mu\text{g/mL}$  has been recommended as a target therapeutic concentration in humans (McEvoy, 1991). A serum concentration of 0.2–0.4  $\mu\text{g/mL}$  of S-ketoprofen is required for the maximum anti-inflammatory effect in adjuvant-induced arthritis in rats (Jamali and Brocks, 1990), whereas at least 1  $\mu\text{g/mL}$  of total ketoprofen is needed to alleviate pain in orthopaedic human patients (Kohler et al., 1985). In our study, the target therapeutic S-ketoprofen plasma concentration of 0.2–0.4  $\mu\text{g/mL}$  was achieved for more than 12 hours after po administration of racemic ketoprofen at a dose of 4 mg/kg, and over 10 hours after im administration at a dose of 3 mg/kg. The concentration of 1.6  $\mu\text{g/mL}$  of R-ketoprofen was only achieved for two hours with these administration routes. Mean total ketoprofen plasma concentrations were at least 1  $\mu\text{g/mL}$  for 10 hours after po and im administration at a dose rate of 3 mg/kg, over 12 hours after po administration at a dose of 6 mg/kg and 8 hours after iv administration at a dose of 3 mg/kg. However, the possible stereoselectivity in plasma protein binding might affect the plasma-tissue ratio, and thus the effective plasma concentrations. Ketoprofen was present for longer in inflammatory exudates than in plasma (Landoni and Lees, 1995b; Landoni et al., 1995a; Landoni et al., 1995b; Landoni et al., 1999; Arifah et al., 2001), and the clinical effect may therefore last longer than estimated from plasma concentrations. In contrast, plasma concentrations may not always correlate well with clinical signs, because the clinical effects are associated more with tissue concentrations. Fosse et al. (2011b) reported a biphasic analgesic effect in piglets; an initial comprehensive but short analgesia followed by a moderate but more sustained analgesia. The authors hypothesized that the short potent analgesia was due the action of R-ketoprofen, while S-ketoprofen was responsible for the longer-lasting moderate analgesia.

### 9.3 Efficacy in the treatment of endotoxemia

Ketoprofen significantly reduced the rectal temperature at doses of 2 and 4 mg/kg, but not at the two lower doses (0.5 and 1 mg/kg). Ketoprofen treatment also significantly lowered the mean plasma TXB<sub>2</sub> concentrations and total clinical scores compared to the control group. Our study indicates that oral administration of ketoprofen could be effective in the treatment of endotoxemia in pigs. Both 2 and 4 mg/kg were shown to be efficacious as a dose, with no significant difference between them. Thus, 2 mg/kg can be considered a sufficient oral dose of ketoprofen for treating endotoxemia in pigs, as increasing the dose above this did not further increase the effect. However, in an earlier study in piglets, ED<sub>50</sub> was found to be 2.5 mg/kg im, suggesting that a higher dose might be beneficial in some cases (Fosse et al., 2011b).

In our study, rectal temperatures and plasma TXB<sub>2</sub> concentrations were elevated for up to 4 hours after endotoxin (4 µg/kg) challenge in control pigs, thus confirming the proper functioning of the model (Schmidt and Banting, 2000; Banting et al., 2003). Although ketoprofen treatment was efficient compared to placebo, no significant differences could be detected between different dose rates with respect to the total clinical score and plasma TXB<sub>2</sub> concentrations. In an earlier study, meloxicam treatment failed to detect significant differences compared to placebo in the total clinical response when pigs were endotoxin challenged at a dose of 4 µg/kg (Friton et al., 2006). At a higher dose of endotoxin (6 µg/kg), there was a greater clinical response in the placebo-treated pigs, and the total clinical score was significantly lower in the meloxicam-treated group compared to placebo treatment (Friton et al., 2006). We might also have detected more pronounced differences between the groups if we had used a higher endotoxin dose, but on the other hand this might have caused more undesirable side effects.

A high TXB<sub>2</sub> concentration before administration of ketoprofen, suggesting a potent acute response to endotoxin, was associated with more severe clinical signs, regardless of the ketoprofen dose. The TXB<sub>2</sub> concentration has been reported to markedly increase, followed by a rapid decline, with the peak occurring within 20 min of endotoxin administration in pigs (Webb et al., 1981; Ball et al., 1986). In our study, the peak occurred within 30 min and there was an obvious decline by 60 min after endotoxin exposure, although the mean concentrations of all the groups were still higher than the baseline, and remained high in the control group up to 4 hours post-challenge.

In our study, the mean plasma TXB<sub>2</sub> concentrations were reduced even at the low ketoprofen plasma concentrations determined in the low dose groups, hence confirming previous findings of effective inhibition of TXB<sub>2</sub> production (Landoni and Lees, 1995a; Landoni et al., 1999; Arifah et al., 2001). Rectal temperatures were also reduced, although the measured ketoprofen plasma concentrations were low. This supports previous findings of a good antipyretic efficacy of ketoprofen in pigs (Arnaud and Consalvi, 1994; Swinkels et al., 1994; Salichs et al., 2012).

The sampling point chosen for determination of the ketoprofen plasma concentration of endotoxin challenged pigs was based on study I, in which the maximum plasma concentration was detected approximately 1 hour after oral administration of ketoprofen in healthy pigs. In the endotoxin challenge study, plasma concentrations in most pigs at this sampling point were much

lower than in our previous study in healthy pigs administered similar doses, and the individual variation was considerable. As we did not investigate the pharmacokinetics of ketoprofen in these endotoxin-challenged pigs, the precise reason for this variability remains unknown. However, the absorption, distribution, metabolism and excretion of drugs may be altered by endotoxin exposure (Yang and Lee, 2008). The maximum plasma concentration had possibly not been reached at the time of sampling. Gastric emptying and gastrointestinal absorption may be delayed in endotoxemia (Hurwitz et al., 1975; Haque et al., 1997). On the other hand, the absorption of drugs could be reduced due to the development of diarrhoea (Yang and Lee, 2008). In dairy cows, the response to oral ketoprofen treatment in endotoxic mastitis was as rapid and effective as that to intramuscular treatment, despite decreased rumen motility after challenge and before treatment (Banting et al., 2008). In our study, the dose was administered by syringe into the mouth, not with a tube into the stomach, to reflect clinical practice. Therefore, some spillage could have occurred, although no notable spilling was observed. As absorption may be reduced and delayed in endotoxemic animals, and variation in ketoprofen plasma concentrations between individual animals could be considerable, it might be advisable to use the parenteral administration route at least in animals with severe endotoxemia (Hurwitz et al., 1975; Choi et al., 2007).

#### 9.4 Efficacy in the treatment of lameness

Our study demonstrated that oral ketoprofen was efficient in alleviating the signs of non-infectious lameness in sows and gilts. The treatment success rates agreed with previous results using meloxicam in lame pigs (Friton et al., 2003). In our study, there was no difference in efficacy between 2 mg/kg and 4 mg/kg doses. The smaller dose was cheaper, easier to administer and may have caused a smaller risk of adverse effects. The findings support the use of oral ketoprofen at 2 mg/kg for up to 5 consecutive days in treating locomotor disorders in pigs.

Diagnosing non-infectious lameness in sows by clinical examination is difficult. In most cases, post-mortem examinations are needed to correctly diagnose the cause of lameness (Dewey et al., 1993; Engblom et al., 2008). In our study, the cause of non-infectious lameness was not determined and diagnosis was based on a thorough clinical examination and analysis of blood samples. Any indication for antibiotics prior to, during or after the treatment was an exclusion criterion. The leukocyte count, haemoglobin and haptoglobin concentrations and PCV values were lower in healthy pigs than in those with an inflammatory process (Odink et al., 1990). In the present study, blood values were within the reference ranges previously reported for healthy pigs (Friendship et al., 1984; Heinonen et al., 2006), confirming the non-infectious nature of the lameness.

Ketoprofen treatment proved to be more efficient on farms with solid concrete or partly slatted floors than on farms with fully slatted floors. The prevalence of lameness was greater among pigs housed on fully slatted floors than on solid floors (Jorgensen, 2003), but no previous studies have been conducted on the effect of floor type on treatment efficacy. Deep bedding reduced the risk of abnormal gait (Kilbride et al., 2009). Due to the limited number of animals in this study, no statistical analysis was performed to evaluate the influence of deep litter bedding on treatment success. However, providing a lame animal with a sufficient amount of bedding should improve the

tolerance of pain and was therefore considered helpful as an adjunct to the use of analgesics in pain management (Short, 1998).

#### 9.4 Tolerance

Undesirable side effects of ketoprofen are typical for non-selective NSAIDs, with gastrointestinal irritation being the most frequently found adverse effect (Wallace 1997). The risk increases as the plasma concentration of the active ingredient increases. Although the medication period was longer (in study IV) and dose rates higher (in all studies) than the approved duration and dose rate, no adverse events caused by use of ketoprofen were clinically observed. However, non-clinical adverse effects were detected in necropsy. Six pigs had ulcers in the mucosa in the glandular part, which indicated gastrointestinal irritation due to ketoprofen. In humans, ulcers in the stomach or small intestine occur in up to 40% of patients taking NSAIDs, although most of these ulcers can only be found by endoscopy because they do not cause symptoms (Hawkey et al., 2000; Laine et al., 2004). Gastric ulceration is also a frequent problem in horses, in which the clinical signs are variable and non-specific. Alternatively, horses may be asymptomatic (Bell et al., 2012). Horses with pre-existing glandular lesions were affected by ketoprofen treatment, and an increase in the number and severity of lesions was observed after ketoprofen administration (MacAllister et al., 1993). Dogs tend to have gastrointestinal irritation after ketoprofen medication, although the lesions were reported to heal after administration was discontinued and were not therefore considered clinically relevant in healthy dogs (Narita et al., 2005). Tolerance studies are usually performed in healthy animals. As anorexia and dehydration are possible risk factors for adverse events of NSAIDs, supportive care should be taken into consideration when medicating diseased animals.

Although the endotoxin dose used in study III was moderate, two pigs spontaneously died, despite treatment with a low dose (1 mg/kg) of ketoprofen. Higher endotoxin doses of 10–20 µg/kg have been used in pigs with no reports of mortality (De Saedeleer et al., 1992; Myers et al., 2010). The cause of the deaths remained uncertain. Fatty degeneration of the liver of one of the dead pigs might have been, at least to some extent, the underlying cause. The post-mortem examination revealed no findings that would have seem to be caused by the use of ketoprofen itself.

Lame animals often require NSAID medication for several days. In our study, the efficacy of oral ketoprofen was comparable with that of injectable meloxicam in a previous study (Friton et al., 2003). Although injectable ketoprofen and meloxicam are not as irritating to the tissues as some other NSAIDs, they do cause local tissue damage after im injection (Pyorala et al., 1999; Magyan and Glávits, 2007). Oral administration is therefore less painful and animal friendly for longer-term medication. However, the long-term side effects of the treatment need to be elucidated. Lameness is also a common problem in piglets, and it indicates pain and might reduce growth rates (Zoric et al., 2003). However, further pharmacological studies are required before recommendations for the use of oral ketoprofen in piglets can be made, since there may be differences in pharmacokinetics in neonatal pigs compared to older ones. Fosse et al. (2011a) reported a higher clearance rate and larger volume of distribution in 6-day-old than 21-day-old pigs.

## 10. CONCLUSIONS

1. S-ketoprofen is the predominant enantiomer in pig plasma after the administration of racemic ketoprofen via oral and intramuscular routes. The relative bioavailability of S-ketoprofen was higher than for R-ketoprofen after oral administration.
2. After administration of racemic ketoprofen at dose rates of 3 mg/kg or 6 mg/kg po, and 3 mg/kg im, bioequivalence could not be detected.
3. An oral dose of 2 mg/kg racemic ketoprofen could be considered as appropriate for treating induced endotoxemia in pigs.
4. Spontaneous non-infectious lameness of sows and gilts could be efficiently treated with racemic oral ketoprofen at a dose rate of 2 mg/kg for five consecutive days.

## 11. ACKNOWLEDGEMENTS

The studies were carried out at the Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki. The financial support provided by the Academy of Finland, the Finnish Veterinary Foundation, the Orion-Farmos Research Foundation and Vetcare Ltd is gratefully acknowledged.

This project would not have been completed without significant help and support from many people. I owe my sincere thanks to everyone who contributed to this work. I owe my sincere gratitude to my supervisors, Professor Outi Vainio, Docent Marja Raekallio, Professor Mari Heinonen and Professor Olli Peltoniemi. You all supported and encouraged me throughout this project. I thank Outi for offering me this opportunity, trusting me and giving me the freedom to do it according to my own schedule. I sincerely thank Marja, especially for all the support and guidance in writing and insightful comments that greatly improved my manuscripts. I am grateful to Mari and Olli for all your great knowledge of pigs and pig production that you shared with me.

The reviewers of this thesis, Professor Birgit Ranheim and Professor Leo van Leengoed, are greatly acknowledged for the invaluable comments and suggestions that improved this thesis. Roy Siddall is warmly thanked for revision of the English text. Professor Dominiek Maes is kindly thanked for accepting the role of opponent in my thesis dissertation.

I wish to thank all my co-authors. Anneli Niemi and Mari Palviainen are deeply thanked for all the work and advice on ketoprofen analysis. My sincere thanks go to Marikki Peltoniemi and Mia Siven for all the pharmacokinetic guidance and analysis. I would not have been able to carry out all the data collection without help from Jussi Honkavaara, Alan Banting and Eve Ala-Kurikka: thank you for your invaluable help. I deeply thank Toomas Orro for guidance, patience and support in the laboratory work on haptoglobins.

I owe my sincere thanks to all the veterinarians at the production Animal Hospital, the veterinary students Marja-Liisa and Tuija, and all the animal nurses who helped me with this project. All the farm owners who helped us to carry out our field study are warmly acknowledged.

I am grateful to Teppo Huttunen and Jouni Junnila, 4Pharma, for their invaluable help with statistics. Arja Heiskanen, Yhtyneet Medix Laboratoriot Oy, is warmly thanked for ketoprofen analysis and Porsolt & Partners for the experimental phase of the endotoxin challenge study. I owe my sincere thanks to Päivi and Kalevi, Vetcare, for letting me use the data and samples collected during Vetcare's studies and offering me a chance to work part-time so I was able to study.

I warmly thank my dear friends Hanna, Taina, Shea and Katja. Thank you for your encouragement, support and friendship.



My warmest thanks are due to my parents and brother. You have always encouraged and supported me.

Finally, I owe my deepest gratitude to my family for their love, patience and support. My three sons, Samu, Joni and Juho, and my husband Paulus, you are the very best thing in my life.

## 12. REFERENCES

- Aberg, G., Ciofalo, V.B., Pendleton, R.G., Ray, G., Weddle, D., 1995. Inversion of (R)- to (S)-ketoprofen in eight animal species. *Chirality* 7, 383-387.
- Al Katheeri, N.A., Wasfi, I.A., Lambert, M., Saeed, A., Khan, I.A., 2000. Pharmacokinetics of ketoprofen enantiomers after intravenous administration of racemate in camels: effect of gender. *Journal of veterinary pharmacology and therapeutics* 23, 137-143.
- Alarcon de la Lastra, C., Nieto, A., Martin, M.J., Cabre, F., Herrerias, J.M., Motilva, V., 2002. Gastric toxicity of racemic ketoprofen and its enantiomers in rat: oxygen radical generation and COX-expression. *Inflammation research* 51, 51-57.
- Alsemgeest, S.P., Kalsbeek, H.C., Wensing, T., Koeman, J.P., van Ederen, A.M., Gruys, E., 1994. Concentrations of serum amyloid-A (SAA) and haptoglobin (HP) as parameters of inflammatory diseases in cattle. *The Veterinary quarterly* 16, 21-23.
- Anil, S.S., Anil, L., Deen, J., 2009. Effect of lameness on sow longevity. *Journal of the American Veterinary Medical Association* 235, 734-738.
- Arifah, A.K., Landoni, M.F., Frea, S.P., Lees, P., 2001. Pharmacodynamics and pharmacokinetics of ketoprofen enantiomers in sheep. *American Journal of Veterinary Research* 62, 77-86.
- Arifah, A.K., Landoni, M.F., Lees, P., 2003. Pharmacodynamics, chiral pharmacokinetics and PK-PD modelling of ketoprofen in the goat. *Journal of veterinary pharmacology and therapeutics* 26, 139-150.
- Arnaud, J.P., Consalvi, P.J., 1994. Assessment of antipyretic activity of ketoprofen in pigs. In: *Proceedings: The 13th International Pig Veterinary Society Congress, Bangkok, Thailand*, pp 351.
- Backstrom, L., 1973. Environment and animal health in piglet production. A field study of incidences and correlations. *Acta veterinaria Scandinavica. Supplementum*, 1-240.
- Backstrom, L., Morkoc, A.C., Connor, J., Larson, R., Price, W., 1984. Clinical study of mastitis-metritis-agalactia in sows in Illinois. *Journal of the American Veterinary Medical Association* 185, 70-73.
- Ball, H.A., Cook, J.A., Wise, W.C., Halushka, P.V., 1986. Role of thromboxane, prostaglandins and leukotrienes in endotoxic and septic shock. *Intensive care medicine* 12, 116-126.
- Banting A, Ripley P, Banting S, Seewald W, Wing M., 2003. A comparison of the effects of *Escherichia coli* endotoxin O55:B5 in conventional French and susceptible Danish pigs treated or not with Econor. *Journal of veterinary pharmacology and therapeutics* 26, 151.

Banting, A., Banting, S., Heinonen, K., Mustonen, K., 2008. Efficacy of oral and parenteral ketoprofen in lactating cows with endotoxin-induced acute mastitis. *Veterinary Record* 163, 506-509.

Bath, G.F., 1998. Management of pain in production animals. *Applied Animal Behaviour Science* 59, 147-156.

Bell, R.J.W., Mogg, T.D., Kingston, J.K., 2012. Equine gastric ulcer syndrome in adult horses: A review. *New Zealand Veterinary Journal* 55, 1-12.

Berry, B.W., Jamali, F., 1991. Presystemic and systemic chiral inversion of R(-)-fenoprofen in the rat. *The Journal of pharmacology and experimental therapeutics* 258, 695-701.

Boothe, D.M., 2001. The analgesic, antipyretic, anti-inflammatory drugs. In Adams HR, edit. *Veterinary pharmacology and therapeutics*. Ames, USA: Iowa State University Press, 2001, 432-449.

Brennan, J.J., Aherne, F.X., 1987. Effect of floor type on the severity of foot lesions and osteochondrosis in swine. *Canadian journal of animal science* 67, 517-523.

Brideau, C., Van Staden, C., Chan, C.C., 2001. In vitro effects of cyclooxygenase inhibitors in whole blood of horses, dogs, and cats. *American Journal of Veterinary Research* 62, 1755-1760.

Cabre, F., Fernandez, F., Zapatero, M.I., Arano, A., Garcia, M.L., Mauleon, D., 1998a. Intestinal ulcerogenic effect of S(+)-ketoprofen in the rat. *Journal of clinical pharmacology* 38, 27S-32S.

Cabre, F., Fernandez, M.F., Calvo, L., Ferrer, X., Garcia, M.L., Mauleon, D., 1998b. Analgesic, antiinflammatory, and antipyretic effects of S(+)-ketoprofen in vivo. *Journal of clinical pharmacology* 38, 3S-10S.

Caldwell, J., Hutt, A.J., Fournel-Gigleux, S., 1988. The metabolic chiral inversion and dispositional enantioselectivity of the 2-arylpropionic acids and their biological consequences. *Biochemical pharmacology* 37, 105-114.

Carabaza, A., Cabre, F., Rotllan, E., Gomez, M., Gutierrez, M., Garcia, M.L., Mauleon, D., 1996. Stereoselective inhibition of inducible cyclooxygenase by chiral nonsteroidal antiinflammatory drugs. *Journal of clinical pharmacology* 36, 505-512.

Carrillo-Jimenez, R., Nurnberger, M., 2000. Celecoxib-induced acute pancreatitis and hepatitis: a case report. *Archives of Internal Medicine* 160, 553-554.

Castro, E., Soraci, A., Fogel, F., Tapia, O., 2000. Chiral inversion of R(-) fenoprofen and ketoprofen enantiomers in cats. *Journal of veterinary pharmacology and therapeutics* 23, 265-271.

Chagnon, M., D'Allaire, S., Drolet, R., 1991. A prospective study of sow mortality in breeding herds. *Canadian journal of veterinary research* 55, 180-184.

Chen, C.Y., Gilbert, C.L., Yang, G.C., Guo, Y.M., SegondsPichon, A., Ma, J.W, Evans, G., Brenig, B., Sargent, C., Affara, N., Huang, L.S., 2008. Maternal infanticide in sows: incidence and behavioural comparisons between savaging and non-savaging sows at parturition. *Applied Animal Behaviour Science* 109, 238-248.

Chitturi, S., George, J., 2002. Hepatotoxicity of commonly used drugs: nonsteroidal anti-inflammatory drugs, antihypertensives, antidiabetic agents, anticonvulsants, lipid-lowering agents, psychotropic drugs. *Seminars in liver disease* 22, 169-183.

Choi, J.S., Jin, M.J., Han, H.K., 2006. Intestinal absorption characteristics of ketoprofen in rats. *Biopharmaceutics & drug disposition* 27, 17-21.

Choi, Y.H., Lee, I., Lee, M.G., 2007. Effects of bacterial lipopolysaccharide on the pharmacokinetics of metformin in rats. *International journal of pharmaceutics* 337, 194-201.

Cox, J.W., Cox, S.R., VanGiessen, G., Ruwart, M.J., 1985. Ibuprofen stereoisomer hepatic clearance and distribution in normal and fatty in situ perfused rat liver. *The Journal of pharmacology and experimental therapeutics* 232, 636-643.

D'Allaire, S., Stein T.E., Leman A.D., 1987. Culling patterns in selected Minnesota swine breeding herds. *Canadian journal of veterinary research* 51, 506-512.

de Beaurepaire, R., Suaudeau, C., Chait, A., Cimetiere, C., 1990. Anatomical mapping of brain sites involved in the antinociceptive effects of ketoprofen. *Brain research* 536, 201-206.

De Saedeleer, V., Wechsung, E., Houvenaghel, A., 1992. Influence of indomethacin on endotoxin-induced changes in gastrointestinal myoelectrical activity and some haematological and clinical parameters in the conscious piglet. *Veterinary research communications* 16, 59-67.

Devleeschouwer, V., Roelandts, R., Garmyn, M., Goossens, A., 2008. Allergic and photoallergic contact dermatitis from ketoprofen: results of (photo) patch testing and follow-up of 42 patients. *Contact dermatitis* 58, 159-166.

Dewey, C.E., Friendship, R.M., Wilson, M.R., 1993. Clinical and postmortem examination of sows culled for lameness. *Canadian Veterinary Journal* 34, 555-556.

Diaz-Reval, M.I., Ventura-Martinez, R., Deciga-Campos, M., Terron, J.A., Cabre, F., Lopez-Munoz, F.J., 2004. Evidence for a central mechanism of action of S-(+)-ketoprofen. *European journal of pharmacology* 483, 241-248.

Diez-Dacal, B., Perez-Sala, D., 2010. Anti-inflammatory prostanoids: focus on the interactions between electrophile signaling and resolution of inflammation. *The Scientific World Journal* 10, 655-675.

Dinchuk, J.E., Car, B.D., Focht, R.J., Johnston, J.J., Jaffee, B.D., Covington, M.B., Contel, N.R., Eng, V.M., Collins, R.J., Czerniak, P.M., 1995. Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II. *Nature* 378, 406-409.

Doster, A.R., 2000. Porcine gastric ulcer. *The Veterinary clinics of North America. Food animal practice* 16, 163-174.

Dubois, N., Lopicque, F., Abiteboul, M., Netter, P., 1993. Stereoselective protein binding of ketoprofen: effect of albumin concentration and of the biological system. *Chirality* 5, 126-134.

Elmore, R.G., Martin, C.E., Berg, J.N., 1978. Absorption of *Escherichia coli* endotoxin from the mammary glands and uteri of early postpartum sows and gilts. *Theriogenology* 10, 439-446.

EMA, 2001. Guidelines for the conduct of bioequivalence studies for veterinary medicinal products.  
[http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Scientific\\_guideline/2009/10/WC500004304.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004304.pdf). Accessed 09/23 2010.

EMA, 1996. Ketoprofen (extension to pigs) Summary report.  
<http://www.emea.europa.eu/pdfs/vet/mrls/007696en.pdf>. Accessed 05.05 2010.

Engblom, L., Eliasson-Selling, L., Lundeheim, N., Belak, K., Andersson, K., Dalin, A.M., 2008. Post mortem findings in sows and gilts euthanised or found dead in a large Swedish herd. *Acta Veterinaria Scandinavica* 50, 25.

Fink, M.P., Rothschild, H.R., Deniz, Y.F., Wang, H.L., Lee, P.C., Cohn, S.M., 1989. Systemic and mesenteric O<sub>2</sub> metabolism in endotoxic pigs: effect of ibuprofen and meclufenamate. *Journal of applied physiology* 67, 1950-1957.

Fosse, T.K., Horsberg, T.E., Haga, H.A., Hormazabal, V., Ranheim, B., 2011a. Enantioselective pharmacokinetics of ketoprofen in piglets: the significance of neonatal age. *Journal of veterinary pharmacology and therapeutics* 34, 153-159.

Fosse, T.K., Toutain, P.L., Spadavecchia, C., Haga, H.A., Horsberg, T.E., Ranheim, B., 2011b. Ketoprofen in piglets: enantioselective pharmacokinetics, pharmacodynamics and PK/PD modelling. *Journal of veterinary pharmacology and therapeutics* 34, 338-349.

Foster, R.T., Jamali, F., 1988. Stereoselective pharmacokinetics of ketoprofen in the rat. Influence of route of administration. *Drug metabolism and disposition* 16, 623-626.

Foster, R.T., Jamali, F., Russell, A.S., Alballa, S.R., 1988a. Pharmacokinetics of ketoprofen enantiomers in healthy subjects following single and multiple doses. *Journal of pharmaceutical sciences* 77, 70-73.

Foster, R.T., Jamali, F., Russell, A.S., Alballa, S.R., 1988b. Pharmacokinetics of ketoprofen enantiomers in young and elderly arthritic patients following single and multiple doses. *Journal of pharmaceutical sciences* 77, 191-195.

Friendship, R.M., Lumsden, J.H., McMillan, I., Wilson, M.R., 1984. Hematology and biochemistry reference values for Ontario swine. *Canadian Journal of Comparative Medicine* 48, 390-393.

Friton, G., Philipp, H., Schneider, T., Kleemann, R., 2003. Investigation on the clinical efficacy and safety of meloxicam (MetacamReg.) in the treatment of non-infectious locomotor disorders in pigs. *Berliner und Munchener Tierarztliche Wochenschrift* 116, 421-426.

Friton, G.M., Schmidt, H., Schrod, W., 2006. Clinical and anti-inflammatory effects of treating endotoxin-challenged pigs with meloxicam. *Veterinary Record* 159, 552-557.

Geisslinger, G., Menzel, S., Wissel, K., Brune, K., 1995. Pharmacokinetics of ketoprofen enantiomers after different doses of the racemate. *British journal of clinical pharmacology* 40, 73-75.

Ghezzi, P., Melillo, G., Meazza, C., Sacco, S., Pellegrini, L., Asti, C., Porzio, S., Marullo, A., Sabbatini, V., Caselli, G., Bertini, R., 1998. Differential contribution of R and S isomers in ketoprofen anti-inflammatory activity: role of cytokine modulation. *The Journal of pharmacology and experimental therapeutics* 287, 969-974.

Granero, G.E., Amidon, G.L., 2008. Possibility of enterohepatic recycling of ketoprofen in dogs. *International journal of pharmaceutics* 349, 166-171.

Grosser, T., Yu, Y., Fitzgerald, G.A., 2010. Emotion recollected in tranquility: lessons learned from the COX-2 saga. *Annual Review of Medicine* 61, 17-33.

Grubb, N.G., Rudy, D.W., Brater, D.C., Hall, S.D., 1999. Stereoselective pharmacokinetics of ketoprofen and ketoprofen glucuronide in end-stage renal disease: evidence for a 'futile cycle' of elimination. *British journal of clinical pharmacology* 48, 494-500.

Hall, S.D., Hassanzadeh-Khayyat, M., Knadler, M.P., Mayer, P.R., 1992. Pulmonary inversion of 2-arylpropionic acids: influence of protein binding. *Chirality* 4, 349-352.

Haque, S.M., Chen, K., Usui, N., Iiboshi, Y., Okuyama, H., Masunari, A., Nezu, R., Takagi, Y., Okada, A., 1997. Effects of endotoxin on intestinal hemodynamics, glutamine metabolism, and function. *Surgery today* 27, 500-505.

Hawkey, C., Laine, L., Simon, T., Beaulieu, A., Maldonado-Cocco, J., Acevedo, E., Shahane, A., Quan, H., Bolognese, J., Mortensen, E., 2000. Comparison of the effect of rofecoxib (a cyclooxygenase 2 inhibitor), ibuprofen, and placebo on the gastroduodenal mucosa of patients with osteoarthritis: a randomized, double-blind, placebo-controlled trial. *Arthritis and Rheumatism* 43, 370-377.

Heinonen, M., Oravainen, J., Orro, T., Seppälä, L., Alakurikka, E., Virolainen, J., Tast, A., Peltoniemi, O.A.T., 2006. Lameness and fertility of sows and gilts in randomly selected loose-housed herds in Finland. *Veterinary Record* 159, 383-387.

Herrero, J.F., Parrado, A., Cervero, F., 1997. Central and peripheral actions of the NSAID ketoprofen on spinal cord nociceptive reflexes. *Neuropharmacology* 36, 1425-1431.

Hersh, E.V., Moore, P.A., Ross, G.L., 2000. Over-the-counter analgesics and antipyretics: a critical assessment. *Clinical therapeutics* 22, 500-548.

Hill, M.A., 1990. Economic relevance, diagnosis, and countermeasures for degenerative joint disease (osteoarthritis) and dyschondroplasia (osteochondrosis) in pigs. *Journal of the American Veterinary Medical Association* 197, 254-259.

Hinton, L.E., McLoughlin, M.A., Johnson, S.E., Weisbrode, S.E., 2002. Spontaneous gastroduodenal perforation in 16 dogs and seven cats (1982-1999). *Journal of the American Animal Hospital Association* 38, 176-187.

Hirsch, A.C., Philipp, H., Kleemann, R., 2003. Investigation on the efficacy of meloxicam in sows with mastitis-metritis-agalactia syndrome. *Journal of veterinary pharmacology and therapeutics* 26, 355-360.

Hunter, R.P., Isaza, R., Koch, D.E., 2003. Oral bioavailability and pharmacokinetic characteristics of ketoprofen enantiomers after oral and intravenous administration in Asian elephants (*Elephas maximus*). *American Journal of Veterinary Research* 64, 109-114.

Hurwitz, A., Furtado, D., Low, R.R., 1975. The effects of endotoxemia and bacteremia on gastrointestinal drug absorption in mice and rats. *The Journal of pharmacology and experimental therapeutics* 192, 236-241.

Hutt, A.J., Caldwell, J., 1983. The metabolic chiral inversion of 2-arylpropionic acids--a novel route with pharmacological consequences. *The Journal of pharmacy and pharmacology* 35, 693-704.

Igarza, L., Soraci, A., Auza, N., Zeballos, H., 2004. Some pharmacokinetic parameters of R-(-)- and S-(+)-ketoprofen: the influence of age and differing physiological status in dairy cattle. *Veterinary research communications* 28, 81-87.

Igarza, L., Soraci, A., Auza, N., Zeballos, H., 2002. Chiral inversion of (R)-ketoprofen: influence of age and differing physiological status in dairy cattle. *Veterinary research communications* 26, 29-37.

Irish Medicines Board, 2010. SPC Document for Dinalgen 300 mg/ml Oral Solution for Use in Drinking Water for Cattle and Pigs.

[http://www.imb.ie/images/uploaded/swedocuments/LicenseSPC\\_10546-001-001\\_26112010141608.pdf](http://www.imb.ie/images/uploaded/swedocuments/LicenseSPC_10546-001-001_26112010141608.pdf). Accessed 20.01 2011.

Jamali, F., Brocks, D.R., 1990. Clinical pharmacokinetics of ketoprofen and its enantiomers. *Clinical pharmacokinetics* 19, 197-217.

Jaussaud, P., Bellon, C., Besse, S., Courtot, D., Delatour, P., 1993. Enantioselective pharmacokinetics of ketoprofen in horses. *Journal of veterinary pharmacology and therapeutics* 16, 373-376.

Jerussi, T.P., Caubet, J.F., McCray, J.E., Handley, D.A., 1998. Clinical endoscopic evaluation of the gastroduodenal tolerance to (R)- ketoprofen, (R)- flurbiprofen, racemic ketoprofen, and paracetamol: a randomized, single-blind, placebo-controlled trial. *Journal of clinical pharmacology* 38, 19S-24S.

Jorgensen, B., 2003. Influence of floor type and stocking density on leg weakness, osteochondrosis and claw disorders in slaughter pigs. *Animal Science* 77, 439-449.

Junot, S., Troncy, É., Keroack, S., Gauvin, D., Castillo, J.R.E., Boivin, R., Bonnet, J.M., 2008. Renal effect of meloxicam versus ketoprofen in anaesthetized pseudo-normovolaemic piglets. *Canadian journal of physiology and pharmacology* 86, 55-63.

Khan, K.N., Venturini, C.M., Bunch, R.T., Brassard, J.A., Koki, A.T., Morris, D.L., Trump, B.F., Maziasz, T.J., Alden, C.L., 1998. Interspecies differences in renal localization of cyclooxygenase isoforms: implications in nonsteroidal antiinflammatory drug-related nephrotoxicity. *Toxicologic pathology* 26, 612-620.

Kilbride, A.L., Gillman, C.E., Green, L.E., 2009. A cross-sectional study of the prevalence of lameness in finishing pigs, gilts and pregnant sows and associations with limb lesions and floor types on commercial farms in England. *Animal Welfare* 18, 215-224.

Kirk, R.K., Svensmark, B., Ellegaard, L.P., Jensen, H.E., 2005. Locomotive disorders associated with sow mortality in Danish pig herds. *Journal of veterinary medicine* 52, 423-428.

Kohler, G., Primbs, P., Morand, J., Rubelt, C., 1985. Correlation between ketoprofen plasma levels and analgesic effect in acute lumbar pain and radicular pain. *Clinical rheumatology* 4, 399-404.



Kokki, H., 2010. Ketoprofen pharmacokinetics, efficacy, and tolerability in pediatric patients. *Paediatric drugs* 12, 313-329.

Kroneman, A., Vellenga, L., van der Wilt, F.J., Vermeer, H.M., 1993. Review of health problems in group-housed sows, with special emphasis on lameness. *The Veterinary Quarterly* 15, 26-29.

Lagrange, F., Penhourcq, F., Matoga, M., Bannwarth, B., 2000. Binding of ketoprofen enantiomers in various human albumin preparations. *Journal of pharmaceutical and biomedical analysis* 23, 793-802.

Laine, L., Maller, E.S., Yu, C., Quan, H., Simon, T., 2004. Ulcer formation with low-dose enteric-coated aspirin and the effect of COX-2 selective inhibition: a double-blind trial. *Gastroenterology* 127, 395-402.

Landoni, M.F., Comas, W., Mucci, N., Anglarilli, G., Bidal, D., Lees, P., 1999. Enantiospecific pharmacokinetics and pharmacodynamics of ketoprofen in sheep. *Journal of veterinary pharmacology and therapeutics* 22, 349-359.

Landoni, M.F., Cunningham, F.M., Lees, P., 1995a. Comparative pharmacodynamics of flunixin, ketoprofen and tolfenamic acid in calves. *The Veterinary record* 137, 428-431.

Landoni, M.F., Cunningham, F.M., Lees, P., 1995b. Pharmacokinetics and pharmacodynamics of ketoprofen in calves applying PK/PD modelling. *Journal of veterinary pharmacology and therapeutics* 18, 315-324.

Landoni, M.F., Lees, P., 1996. Pharmacokinetics and pharmacodynamics of ketoprofen enantiomers in the horse. *Journal of veterinary pharmacology and therapeutics* 19, 466-474.

Landoni, M.F., Lees, P., 1995a. Comparison of the anti-inflammatory actions of flunixin and ketoprofen in horses applying PK/PD modelling. *Equine veterinary journal* 27, 247-256.

Landoni, M.F., Lees, P., 1995b. Pharmacokinetics and pharmacodynamics of ketoprofen enantiomers in calves. *Chirality* 7, 586-597.

Landoni, M.F., Lees, P., 1995c. Influence of formulation on the pharmacokinetics and bioavailability of racemic ketoprofen in horses. *Journal of veterinary pharmacology and therapeutics* 18, 446-450.

Landoni, M.F., Soraci, A.L., Delatour, P., Lees, P., 1997. Enantioselective behaviour of drugs used in domestic animals: a review. *Journal of veterinary pharmacology and therapeutics* 20, 1-16.

Larsen, C., Jensen, B.H., Olesen, H.P., 1991. Bioavailability of ketoprofen from orally administered ketoprofen-dextran ester prodrugs in the pig. *Acta Pharmaceutica Nordica* 3, 71-76.

Lascelles, B.D., McFarland, J.M., Swann, H., 2005. Guidelines for safe and effective use of NSAIDs in dogs. *Veterinary Therapeutics* 6, 237-251.

Lees, P., Landoni, M.F., Giraudel, J., Toutain, P.L., 2004. Pharmacodynamics and pharmacokinetics of nonsteroidal anti-inflammatory drugs in species of veterinary interest. *Journal of veterinary pharmacology and therapeutics* 27, 479-490.

Lees, P., Taylor, P.M., Landoni, F.M., Arifah, A.K., Waters, C., 2003. Ketoprofen in the cat: pharmacodynamics and chiral pharmacokinetics. *Veterinary journal* 165, 21-35.

Liggett, A.D., Harrison, L.R., Farrell, R.L., 1986. Acute inflammatory effects of intratracheally instilled *Escherichia coli* endotoxin and sonicated suspension of *Haemophilus pleuropneumoniae* in swine. *Canadian journal of veterinary research* 50, 526-531.

Little, D., Jones, S.L., Blikslager, A.T., 2007. Cyclooxygenase (COX) inhibitors and the intestine. *Journal of Veterinary Internal Medicine*. 21: 3, 367-377.

Luna, S.P., Basilio, A.C., Steagall, P.V., Machado, L.P., Moutinho, F.Q., Takahira, R.K., Brandao, C.V., 2007. Evaluation of adverse effects of long-term oral administration of carprofen, etodolac, flunixin meglumine, ketoprofen, and meloxicam in dogs. *American Journal of Veterinary Research* 68, 258-264.

MacAllister, C.G., Morgan, S.J., Borne, A.T., Pollet, R.A., 1993. Comparison of adverse effects of phenylbutazone, flunixin meglumine, and ketoprofen in horses. *Journal of the American Veterinary Medical Association* 202, 71-77.

Magyan, T., Glávits, R., 2007. Clinical comparison of tissue tolerance of Meloxicam 20 mg injectable and flunixin injectable in pigs. *Pig Journal* 59, 112-124.

Makimura, S., Suzuki, N., 1982. Quantitative determination of bovine serum Haptoglobin and its elevation in some inflammatory diseases. *The Japanese journal of veterinary science* 44, 15-21.

Martineau, G.P., Smith, B.B., Doize, B., 1992. Pathogenesis, prevention, and treatment of lactational insufficiency in sows. *Veterinary Clinics of North America, Food Animal Practice: Swine Reproduction* 8, 661-684.

Masso Gonzalez, E.L., Patrignani, P., Tacconelli, S., Garcia Rodriguez, L.A., 2010. Variability among nonsteroidal antiinflammatory drugs in risk of upper gastrointestinal bleeding. *Arthritis and Rheumatism* 62, 1592-1601.

Mayeux, P.R., 1997. Pathobiology of lipopolysaccharide. *Journal of toxicology and environmental health* 51, 415-435.

McEvoy, G.K., 1991. American Hospital Formulary Service. Drug Information. In: American Society of Hospital Pharmacists Inc., Bethesda, MD, pp. 1107-1112.

McEwen, J., De Luca, M., Casini, A., Gich, I., Barbanoj, M.J., Tost, D., Artigas, R., Mauleon, D., 1998. The effect of food and an antacid on the bioavailability of dexketoprofen trometamol. *Journal of clinical pharmacology* 38, 41S-45S.

Mehvar, R., Jamali, F., 1988. Pharmacokinetic analysis of the enantiomeric inversion of chiral nonsteroidal antiinflammatory drugs. *Pharmaceutical research* 5, 76-79.

Menzel, S., Beck, W.S., Brune, K., Geisslinger, G., 1993. Stereoselectivity of biliary excretion of 2-arylpropionates in rats. *Chirality* 5, 422-427.

Menzel, S., Sauernheimer, C., Brune, K., Geisslinger, G., 1994. Is the inversion from R- to S-ketoprofen concentration dependent? Investigations in rats in vivo and in vitro. *Biochemical pharmacology* 47, 1267-1270.

Miller, S.B., 2006. Prostaglandins in health and disease: an overview. *Seminars in arthritis and rheumatism* 36, 37-49.

Montoya, L., Ambros, L., Kreil, V., Bonafine, R., Albarellos, G., Hallu, R., Soraci, A., 2004. A pharmacokinetic comparison of meloxicam and ketoprofen following oral administration to healthy dogs. *Veterinary research communications* 28, 415-428.

Moore, J.N., Barton, M.H., 2003. Treatment of endotoxemia. *The Veterinary clinics of North America. Equine practice* 19, 681-695.

Myers, M.J., Farrell, D.E., Howard, K.D., Kawalek, J.C., 2010. Effects of intravenous administration of lipopolysaccharide on cytochrome P450 isoforms and hepatic drug metabolizing enzymes in swine. *American Journal of Veterinary Research* 71, 342-348.

Nakamura, Y., Yamaguchi, T., 1987. Stereoselective metabolism of 2-phenylpropionic acid in rat. I. In vitro studies on the stereoselective isomerization and glucuronidation of 2-phenylpropionic acid. *Drug metabolism and disposition* 15, 529-534.

Narita, T., Tomizawa, N., Sato, R., Goryo, M., Hara, S., 2005. Effects of long-term oral administration of ketoprofen in clinically healthy beagle dogs. *The Journal of veterinary medical science* 67, 847-853.

Navarre, C.B., Ravis, W.R., Campbell, J., Nagilla, R., Duran, S.H., Pugh, D.G., 2001. Stereoselective pharmacokinetics of ketoprofen in llamas following intravenous administration. *Journal of veterinary pharmacology and therapeutics* 24, 223-226.

Neirinckx, E., Croubels, S., De Boever, S., Remon, J.P., Bosmans, T., Daminet, S., De Backer, P., Vervaet, C., 2011a. Species comparison of enantioselective oral bioavailability and pharmacokinetics of ketoprofen. *Research in veterinary science* 91, 415-421.

Neirinckx, E., Croubels, S., Remon, J.P., Devreese, M., De Backer, P., Vervaeke, C., 2011b. Chiral inversion of R(-) to S(+) ketoprofen in pigs. *Veterinary journal* 190, 290-292.

Odink, J., Smeets, J.F.M., Visser, I.J.R., Sandman, H., Snijders, J.M.A., 1990. Hematological and clinicochemical profiles of healthy swine and swine with inflammatory processes. *Journal of animal science* 68, 163-170.

Olson, N.C., Brown, T.T., Jr, Anderson, D.L., 1985. Dexamethasone and indomethacin modify endotoxin-induced respiratory failure in pigs. *Journal of applied physiology* 58, 274-284.

Ossipov, M.H., Jerussi, T.P., Ren, K., Sun, H., Porreca, F., 2000. Differential effects of spinal (R)-ketoprofen and (S)-ketoprofen against signs of neuropathic pain and tonic nociception: evidence for a novel mechanism of action of (R)-ketoprofen against tactile allodynia. *Pain* 87, 193-199.

Owen, S.G., Roberts, M.S., Friesen, W.T., 1987. Rapid high-performance liquid chromatographic assay for the simultaneous analysis of non-steroidal anti-inflammatory drugs in plasma. *Journal of chromatography* 416, 293-302.

Palylyk, E.L., Jamali, F., 1992. Gender related differences in the pharmacokinetics of ketoprofen enantiomers in the rat. *Pharmaceutical research* 9, S263.

Panico, A.M., Cardile, V., Gentile, B., Garufi, F., Avondo, S., Ronsisvalle, S., 2005. "In vitro" differences among (R) and (S) enantiomers of profens in their activities related to articular pathophysiology. *Inflammation* 29, 119-128.

Panus, P.C., Ferslew, K.E., Tober-Meyer, B., Kao, R.L., 1999. Ketoprofen tissue permeation in swine following cathodic iontophoresis. *Physical Therapy* 79, 40-49.

Papadopoulos, G.A., Vanderhaeghe, C., Janssens, G.P., Dewulf, J., Maes, D.G., 2010. Risk factors associated with postpartum dysgalactia syndrome in sows. *Veterinary journal* 184, 167-171.

Pinardi, G., Sierralta, F., Miranda, H.F., 2001. Interaction between the antinociceptive effect of ketoprofen and adrenergic modulatory systems. *Inflammation* 25, 233-239.

Pyorala, S., Laurila, T., Lehtonen, S., Leppä, S., Kaartinen, L., 1999. Local tissue damage in cows after intramuscular administration of preparations containing phenylbutazone, flunixin, ketoprofen and metamizole. *Acta Veterinaria Scandinavica* 40, 145-150.

Raekallio, M., Heinonen, K.M., Kuussaari, J., Vainio, O., 2003. Pain alleviation in animals: attitudes and practices of Finnish veterinarians. *The Veterinary Journal* 165, 131-135.

Rainsford, K.D., Stetsko, P.I., Sirko, S.P., Debski, S., 2003. Gastrointestinal mucosal injury following repeated daily oral administration of conventional formulations of indometacin and other

non-steroidal anti-inflammatory drugs to pigs: a model for human gastrointestinal disease. *The Journal of pharmacy and pharmacology* 55, 661-668.

Reiner, G., Hertrampf, B., Richard, H.R., 2009. Dysgalactia in the sow post partum - a review with special emphasis on pathogenesis. *Tierärztliche Praxis* 37, 305-318.

Robertson, I.D., Accioly, J.M., Moore, K.M., Driesen, S.J., Pethick, D.W., Hampson, D.J., 2002. Risk factors for gastric ulcers in Australian pigs at slaughter. *Preventive veterinary medicine* 53, 293-303.

Rudy, A.C., Liu, Y., Brater, C., Hall, S.D., 1998. Stereoselective pharmacokinetics and inversion of (R)- ketoprofen in healthy volunteers. *Journal of clinical pharmacology* 38, 3S-10S.

Salichs, M., Sabate, D., Ciervo, O., Homedes, J., 2012. Comparison of the antipyretic efficacy of ketoprofen, acetylsalicylic acid, and paracetamol, orally administered to swine. *Journal of veterinary pharmacology and therapeutics* 35, 198-201.

Sallustio, B.C., Purdie, Y.J., Whitehead, A.G., Ahern, M.J., Meffin, P.J., 1988. The disposition of ketoprofen enantiomers in man. *British journal of clinical pharmacology* 26, 765-770.

Sattari, S., Jamali, F., 1994. Evidence of absorption rate dependency of ibuprofen inversion in the rat. *Chirality* 6, 435-439.

Savage, T., 2004. Raising pigs at home.

<http://extension.unh.edu/Agric/AGDLEP/docs/pigraise.pdf>. Accessed 08/2010 2010.

Schmidt, H., Banting, A., 2000. Meloxicam (Metacam) for the alleviation of inflammatory conditions in swine- selection of an effective dose. In: *Proceeding: The 16th International Pig Veterinary Society Congress, Melbourne, Australia*, pp. 99.

Schmitt, M., Guentert, T.W., 1990. Biopharmaceutical evaluation of ketoprofen following intravenous, oral, and rectal administration in dogs. *Journal of pharmaceutical sciences* 79, 614-616.

Schrauwen, E., Vandeplasseche, G., Laekeman, G., Houvenaghel, A., 1983. Endotoxin shock in the pig: release of prostaglandins and beneficial effects of flubiprofen. *Archives internationales de pharmacodynamie* 262, 332-334.

Shah, V.P., Midha, K.K., Findlay, J.W., Hill, H.M., Hulse, J.D., McGilveray, I.J., McKay, G., Miller, K.J., Patnaik, R.N., Powell, M.L., Tonelli, A., Viswanathan, C.T., Yacobi, A., 2000. Bioanalytical method validation--a revisit with a decade of progress. *Pharmaceutical research* 17, 1551-1557.

Short, C.E., 1998. Fundamentals of pain perception in animals. *Applied Animal Behaviour Science* 59, 125-133.

Simmonds, R.G., Woodage, T.J., Duff, S.M., Green, J.N., 1980. Stereospecific inversion of (R)-(-)-benoxaprofen in rat and man. *European journal of drug metabolism and pharmacokinetics* 5, 169-172.

Singh, R.D., Sarita Devi, Gondaliya, S.R., Bhavsar, S.K., Thaker, A.M., 2009a. Safety of ketoprofen in cow calves following repeated intravenous administration. *Veterinary World* 2, 105-107.

Smith, B.B., Wagner, W.C., 1984. Suppression of prolactin in pigs by *Escherichia coli* endotoxin. *Science* 224, 605-607.

Soraci, A., Benoit, E., 1995. In vitro fenoprofenyl-coenzyme A thioester formation: interspecies variations. *Chirality* 7, 534-540.

Stanton, M.E., Bright, R.M., 1989. Gastroduodenal ulceration in dogs. Retrospective study of 43 cases and literature review. *Journal of veterinary internal medicine* 3, 238-244.

Stiegler, S., Birkel, M., Jost, V., Lange, R., Lucker, P.W., Wetzelsberger, N., 1995. Pharmacokinetics and relative bioavailability after single dose administration of 25 mg ketoprofen solution as compared to tablets. *Methods and findings in experimental and clinical pharmacology* 17, 129-134.

Streppa, H.K., Jones, C.J., Budsberg, S.C., 2002. Cyclooxygenase selectivity of nonsteroidal anti-inflammatory drugs in canine blood. *American Journal of Veterinary Research* 63, 91-94.

Suesa, N., Fernandez, M.F., Gutierrez, M., Rufat, M.J., Rotllan, E., Calvo, L., Mauleon, D., Carganico, G., 1993. Stereoselective cyclooxygenase inhibition in cellular models by the enantiomers of ketoprofen. *Chirality* 5, 589-595.

Swaby, H., Gregory, N.G., 2012. A note on the frequency of gastric ulcers detected during post-mortem examination at a pig abattoir. *Meat Science* 90, 269-271.

Swinkels, J.M., Pijpers, A., Vernooij, J.C., Van Nes, A., Verheijden, J.H., 1994. Effects of ketoprofen and flunixin in pigs experimentally infected with *Actinobacillus pleuropneumoniae*. *Journal of veterinary pharmacology and therapeutics* 17, 299-303.

Thorup, F., 2000. Effect of treatment for MMA-retrospective observations. In: *Proceedings: The 16th International Pig Veterinary Society Congress, Melbourne, Australia*, pp 99.

Threlfall, W.R., Martin, C.E., 1973. Swine agalactia in Missouri. *Veterinary medicine, small animal clinician* 68, 423.

Tucker, G.T., Lennard, M.S., 1990. Enantiomerspecific pharmacokinetics. *Pharmacology and therapeutics* 45, 309-329.

Upton, R.A., Buskin, J.N., Guentert, T.W., Williams, R.L., Riegelman, S., 1980. Convenient and sensitive high-performance liquid chromatography assay for ketoprofen, naproxen and other allied drugs in plasma or urine. *Journal of chromatography* 190, 119-128.

Vane, J.R., 1971. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature* 231, 232-235.

Verde, C.R., Simpson, M.I., Frigoli, A., Landoni, M.F., 2001. Enantiospecific pharmacokinetics of ketoprofen in plasma and synovial fluid of horses with acute synovitis. *Journal of veterinary pharmacology and therapeutics* 24, 179-185.

Wagner, W.C., 1982. Mastitis-metritis-agalactia. *The Veterinary clinics of North America. Large animal practice* 4, 333-341.

Wallace, J.L., 1997. Nonsteroidal anti-inflammatory drugs and gastroenteropathy: the second hundred years. *Gastroenterology* 112, 1000-1016.

Weary, D., Niel, L., Flower, F.C., Fraser, D., 2006. Identifying and preventing pain in animals. *Applied Animal Behaviour Science* 100, 64-76.

Webb, P.J., Westwick, J., Scully, M.F., Zahavi, J., Kakkar, V.V., 1981. Do prostacyclin and thromboxane play a role in endotoxic shock? *The British journal of surgery* 68, 720-724.

Wechter, W.J., Loughhead, D.G., Reischer, R.J., VanGiessen, G.J., Kaiser, D.G., 1974. Enzymatic inversion at saturated carbon: nature and mechanism of the inversion of R(-) p-iso-butyl hydratropic acid. *Biochemical and biophysical research communications* 61, 833-837.

Yang, K.H., Lee, M.G., 2008. Effects of endotoxin derived from *Escherichia coli* lipopolysaccharide on the pharmacokinetics of drugs. *Archives of Pharmacal Research* 31, 1073-1086.

Yasui, H., Yamaoka, K., Nakagawa, T., 1996. Moment analysis of stereoselective enterohepatic circulation and unidirectional chiral inversion of ketoprofen enantiomers in rat. *Journal of pharmaceutical sciences* 85, 580-585.

Zoric, M., Stern, S., Lundeheim, N., Wallgren, P., 2003. Four-year study of lameness in piglets at a research station. *Veterinary Record* 153, 323-328.

## 13. ORIGINAL ARTICLES