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**Effect of Age on the
Pharmacokinetics of Meloxicam in
ISA Brown Chickens (*Gallus gallus
domesticus*).**

*A thesis presented in partial fulfilment of
the requirements for the degree of*

Master of Science

in

Physiology

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New Zealand.

Megan Gildersleve

2015

Effect of Age on the Pharmacokinetics of Meloxicam in ISA Brown Chickens (*Gallus gallus domesticus*).

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Abstract

The Non-Steroidal Anti-Inflammatory drug (NSAID) meloxicam has been deemed a safe and effective treatment for numerous inflammatory conditions and injuries from extensive pharmacokinetic and pharmacodynamic studies in various mammalian species. However, there is a lack of meloxicam pharmacokinetic information in avian species. This leads to pharmacokinetic data being extrapolated from mammals in order to administer and treat birds. This often leads to ineffective pain relief or overdoses that can be fatal for birds. Due to this void in literature this study was designed to increase the basic pharmacokinetic knowledge in birds but to also determine if age affects the pharmacokinetics of meloxicam in ISA Brown chickens. Meloxicam was injected intravenously (IV) at 2 mg/kg in 20 healthy ISA Brown chickens (*Gallus gallus domesticus*). One group consisted of 10 ISA brown chickens that were 18 weeks old, the second group consisted of 10 ISA Brown chickens that were 24 months old. Serial blood samples were withdrawn from a catheterised vein from each ISA Brown chicken into a heparinised vial at 0, 10, 20, 30 minutes, 1, 4, 8, 10, 12 hours after the administration of meloxicam.

The pharmacokinetics for ISA Brown chickens were calculated using the non-compartmental model, which was analysed using the mean data from each group of ISA Brown

chickens. The elimination half-life, steady state volume of distribution and mean resident time were significantly higher in the 24 month old ISB Brown chickens compared to the 18 week old ISA Brown chickens. Overall, the results indicate that as an ISA Brown chicken ages the pharmacokinetics of meloxicam show some significant changes in crucial pharmacokinetic parameters. The differences in the pharmacokinetic parameters may ultimately affect the efficacy of meloxicam when treating 'geriatric' birds due to possible age-related health issues in the liver and kidneys, which are major organs involved in processing drugs.

KEYWORDS: Meloxicam, non-steroidal anti-inflammatory drugs, ISA Brown chickens, analgesia, pharmacokinetics.

|

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List of abbreviations

- AIC** – Alkaline Information Criterion
- AA** - Arachidonic acid
- AUC** – Area under the curve
- AUMC** – Area under the moment curve
- BIC** – Bayesian Information Criterion
- BMR** – Basal Metabolic Rate
- C_{lb}** – Body (systemic) clearance
- CNS** – Central nervous system
- C_{p0}** – Concentration at time zero
- COX-1** – Cyclooxygenase 1
- COX-2** - Cyclooxygenase 2
- CYP** - Cytochrome P450
- t_{1/2α}** – Distribution half-life
- α** – Distribution rate constant
- DAD** – Diode array detector
- ED** – Electrochemical detector
- t_{1/2β}** – Elimination half-life
- β** – Elimination rate constant
- GI** – Gastrointestinal
- GFR** - Glomerular filtration rate
- t_{1/2}** – Half-life
- HPLC** – High Performance Liquid Chromatography
- IM** – Intramuscular
- IT** – Intrathecal
- IV** – Intravenous
- LC/MS** – Liquid chromatography/Mass spectrophotometer
- LOD** – Limit of detection
- LLE** – Liquid-liquid extraction
- LLD** – Lower limit of detection
- LLQ** – Lower limit of quantification

MAT - Mean absorption time

MEC – Mean effective concentration

MRT – Mean residence time

C_{max} - Maximum concentration

NSAIDs – Non Steroidal Anti-inflammatory drugs

OA – Oral administration

C_p – Plasma drug concentration at any time

SPE – Solid phase extraction

SC – Subcutaneous

TXA₂ and TXB₂ – Thromboxane

C_L - Total clearance

V_{dt}– Total volume of distribution

V_d – Volume of distribution

V_{dc} – Volume of distribution, central compartment

V_{dp} – Volume of distribution, peripheral compartment

V_{ss} – Volume of distribution, steady state

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Chapter One

General Introduction

1. Non-Steroidal Anti-Inflammatory drugs (NSAIDs)

The expression of pain is a necessity in order for veterinarians to identify and treat the cause of pain which will inherently improve the overall welfare of the animal (Mellor, 2008). Part of treating the pain is understanding the mechanisms behind analgesic drugs in both their pharmacokinetic and pharmacodynamic properties. It is the information gathered through these investigations which help to build a better understanding into which analgesic drugs will best treat a variety of painful experiences that may arise.

Non-Steroidal Anti-Inflammatory drugs (NSAIDs) are effective in alleviating pain that arises from the production of prostaglandins. This section will discuss the discovery and history of NSAIDs, along with an in-depth examination of the body's ability to produce prostaglandins, their role when the bodily tissues are damaged and the side effects which are attributed to the consumption of these drugs. In addition, an overview of the use of NSAIDs in birds and the scientific findings in their efficacy in treating injuries, also highlighting the lack of knowledge that is present and the possible negative effects of NSAIDs that can occur in birds. The fourth section of this chapter takes an in-depth look into scientific findings on the pharmacokinetics of meloxicam in various bird species. This analysis produces a platform from which the results found in chapter two can be analysed and conclusions drawn. The final section covers the concept of allometric scaling and the associated issues with extrapolating data between mammals and birds, especially when administering NSAIDs.

1.1. History of NSAIDs

In the history of medicine and drug discovery the use of plants for medicinal use has led to the improvement and efficacy of the drugs that we use today. Analgesic drugs such as aspirin and morphine were first discovered in the bark of the willow tree and in the seeds of the poppy respectively (Mahdi, 2010; Newman, 2000). One of the earliest records of willow bark being used as an analgesic was by the Greek physician Dioscorides around 100 BC. This approach of extracting substances from bark for pain relief was later used by Hildegard von Bingen in continental Europe. During this time these physicians thought local inflammation caused general inflammation which manifested as a fever and malaise. We know now that these generalised symptoms are the result of the production and release of pyrogenic cytokines in particular prostaglandins, and tumour necrosis factor alpha (TNF_{α}) and interleukin-6 contribute to the onset of a fever (Brune & Hinz, 2004).

Aspirin was synthesised and marketed as an anti-inflammatory drug in 1897, the mechanisms behind this were not discovered until 1971. John Vane was the first to investigate the mechanisms of aspirin by comparing the concentrations of aspirin, indomethacin and sodium salicylate in aliquots of the supernatant from a broken cell homogenate from a guinea pig lung which had been incubated with arachidonic acid (Vane, 1971). The results showed that all three drugs had a dose-dependent inhibition on the production of a prostanoid, prostaglandin (PG) (Botting, 2010).

Vane and his colleagues were also able to demonstrate that the prostaglandin that was released was also inhibited in the isolated dog spleen when the animal was administered aspirin or indomethacin. In the same year Smith and Wills found that after volunteers took a 600 mg aspirin orally, aspirin treated blood samples showed platelets did not aggregate, but also released lower levels of prostaglandins compared to the samples that did not contain any aspirin after the platelets were washed and incubated with thrombin. Through these experiments and other scientists' drug discoveries (*Table 1*) a new class of drug was created named non-steroidal anti-inflammatory drugs (NSAIDs). Even though they shared similar analgesic pharmacological properties with drugs like morphine, hydrocortisone and mepyramine, these new drugs were distinguished by their ability to inhibit prostaglandin, unlike any other drug on the market.

In 1976, it was discovered that the production of prostanoids from the biosynthesis pathway of phospholipids involved a homogenous enzyme cyclooxygenase (COX-1). This enzyme is a membrane bound hemo- and glycoprotein, which is primarily found in the endoplasmic reticulum (ER) of prostanoid-forming cells. The discovery of the COX enzyme's existence and impact on the pharmacological management of inflammation was a landmark finding. However, even with this discovery chemists and pharmacists still found that NSAIDs varied in their potency, and their undesired effects remained (Botting, 2010; Marnett & Kalgutkar, 1999).

It was not until 1991 that Needleman discovered, and Harvey Herschman isolated

and characterised, the presence of a second COX (COX-2). Found on a different gene compared to COX-1, COX-2 still shares 60% of the amino acid structure. However, it is primarily expressed in response to inflammatory stimuli which include: bacteria from lipopolysaccharides, and cytokines which include interleukin-1, mitogens and growth factors which are released from macrophages (Botting, 2010). Vane and Needleman thought that COX-2 would produce sufficient anti-inflammatory and analgesic effects, while the toxicity mechanisms of their effect would be eliminated when under the influence of cortisone. This was only partially correct, as drugs that interfered with COX-2 reduced these toxicity effects, but COX-1 was still involved, which showed that COX-2 was expressed and regulated differently to COX-1 (Brune & Hinz, 2004). The role of COX-1 and COX-2 will be further discussed in section 1.2.2.

Table 1: The Discoveries of other early NSAIDs (Brune & Hinz, 2004)

| NSAIDS | Scientists | Discoveries/Improvements |
|--|---|---|
| Phenylbutazone (1940's) | Otto Gsell and Ludwig Heilmeyer | Discovered the anti-inflammatory properties that were not seen in salicylics or phenazones. |
| | Axelrod and Brodie | Compared to phenazones, the plasma concentration-time relationship was greater in phenylbutazone. |
| | Gerhard Wilhelms | Produced the first model of inflammation which was used to define the activity of these drugs, which are now known as NSAIDs through reducing the redness on the skin following irradiation from UV light in guinea pigs. |
| Indomethacin (1963) | Shen | Synthesised this new NSAID. |
| Ibuprofen (1969) and Flurbiprofen (1977) | Boots | Derived from propionic acid. |
| Diclofenac (1974) and Ketoprofen (1973) | Geigy pharmaceuticals, Rhone-Poulenc, and Bayer pharmaceuticals | Derived from acetic acid and propionic acid derivatives. |
| Phenylbutazone and Oxybutazone | Lombardino | Revitalised the concept of ketoenolic acids and improved their potency and pharmaceutical interactions. No further changes were made in the efficacy or side effects. This resulted in the production of other NSAIDs like piroxicam, tenoxicam, and meloxicam. |

1.2. Mechanisms and side effects of NSAIDs

1.2.1. Biosynthesis of prostaglandins

The onset of acute or chronic inflammation is a complex process that requires the initiation and interaction of various mediators, resulting in the 5 cardinal signs of inflammation: swelling, heat, redness, pain and loss of function. These mediators are synthesised de novo or released from storage sites. The cardinal signs are the result of several underlying physiological actions which include: vasodilation of the local blood vessels which increases blood flow to the surrounding tissues; increased permeability of capillaries leading to fluid moving into the interstitial space; increased concentration of fibrinogens and other proteins leaking from the capillaries, which causes the fluid within the interstitial space to clot; and migration of monocytes and granulocytes into the surrounding tissues via chemotaxis, which causes the tissue cells to swell (Hall & Guyton, 2011; Riviere & Papich, 2009).

The precursor to prostaglandin synthesis is an unsaturated 20-carbon fatty acid, found in the cell membranes as a phospholipid ester, known as arachidonic acid (AA). Arachidonic acid is released from the membrane phospholipids by secretory (sPLA₂) or cytoplasmic (cPLA₂) phospholipases, which enables the conversion cascade to begin. The COX enzymes converts AA to prostaglandin G₂ (PGG₂), which converts to an endoperoxide intermediate via peroxidase to prostaglandin H₂ (PGH₂) (Mardini & FitzGerald, 2001). The

transformation of both PGG₂ and PGH₂ occurs relatively quickly as they are unstable and have half-lives of around 5 minutes (Riviere, 2011b). From here on various cell-specific isomerases and synthases produce active prostanoids, that include prostaglandin D₂ (PGD₂), prostaglandin E₂ (PGE₂), prostaglandin F_{2a} (PGF_{2a}), prostacyclin (PGI₂), and thromboxane A₂ (TxA₂). These variations of active prostaglandins and thromboxane go on and act as second messengers which interact with various receptors, including prostanoid G-protein coupled receptors (*Figure 1*) (Rao & Knaus, 2008).

However, all the roles of the various prostaglandins during the inflammatory process are yet to be fully understood. The experience of pain is due to the prostaglandins lowering the threshold of activation of the thermal, mechanical, and chemical nociceptors that relay pain signals up to the higher levels of the central nervous system (CNS). NSAIDs help to increase those thresholds therefore reduces the experience of pain brought on by the tissue injury or inflammation (Machin, 2005a). It is thought that PGE₂ is the predominant PG during the initiation and continual events of the inflammatory process. It has the ability to activate other mediators like bradykinin and histamine, modify the adaptive immune system, in particular the B and T cell functions, along with the ability to inhibit the secretion of interleukin-2 (IL-2) (Boothe, 2000).

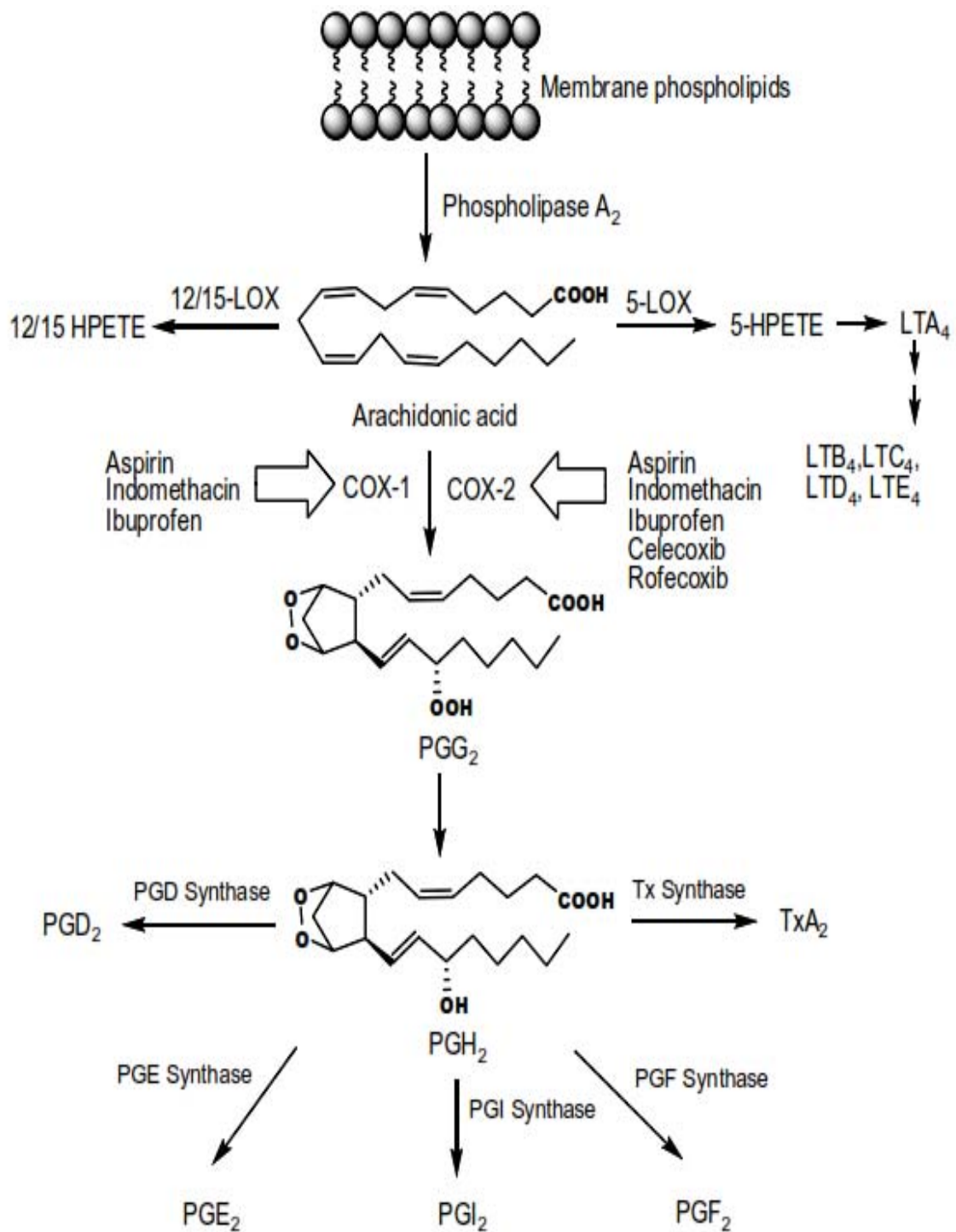


Figure 1: The biosynthesis pathway of the various prostaglandins (PG) from arachidonic acid (AA). The inhibition of COX-1 and COX-2 from the NSAIDs aspirin, indomethacin, ibuprofen, rofecoxib, and celecoxib results in the inhibition of prostaglandins (Rao & Knaus, 2008).

1.2.2. COX-1 and COX-2

The COX-1 and COX-2 are homodimeric enzymes that are embedded into a single layer of the lipid bilayer of the plasma cell membranes. Their overall structures are similar including their enzymatic active site, which is SER530 (referred to as SER516 in COX-2 due to a small structural change). As a result, the only difference is in their response to AA and NSAIDs (*Figure 2*). For example, when aspirin is present it binds to COX-1, strongly inhibiting Ser530 through acetylation and weakly binding to Arg120, and this prevents AA from binding with Tyr385 which is on the apex of the protein. In contrast, aspirin still binds to Ser516 through acetylation, but the active site of COX-2 is slightly larger with the addition of a side pocket, which allows AA to get past creating 15-R-hydroxyeicosatetraenoic acid (15-R-HETE) (Botting, 2010).

During the inflammatory process the difference between COX-1 and COX-2 become evident. COX-1 is a constitutive enzyme that can be found in most of the body's tissues, and therefore the expression is constant regardless of whether it is being expressed in normal tissues (Marnett & Kalgutkar, 1999). For example, the expression of COX-1 only increases 2-3 fold in tissues that have been injured, compared to the 20-fold baseline increase in the expression of COX-2. Unlike COX-1, COX-2 is an inducible enzyme that is only found in certain tissues and is synthesised and up-regulated when macrophages and other inflammatory cells are activated in tissues that are injured or inflamed. The result of increased COX-2 within these injured cells is the increased production of prostanoids, which

amplify the nociceptive input and transmission of these signals to the peripheral nervous system (PNS) and CNS. Through these mechanisms COX-2 has been found to be the main contributor of the pain and hyperalgesia experienced after tissue injury (Mathews, 2002).

The inhibition of COX-1 through NSAIDs is likely to inhibit or reduce the primary plug formation of platelets, the vascular tone of the kidney, and the cytoprotective function of the gastric mucosa, whereas COX-2 seems to have protective mechanisms over the gastrointestinal tract. Furthermore, it appears that COX-2 has constitutive functions that are associated with the functions of nerves, brain, ovaries, bone metabolism and kidneys. This raises the concern with the COX-2 inhibiting NSAIDs and their possible adverse side effects on these particular organs (*Figure 3*) (Marnett & Kalgutkar, 1999; Mathews, 2002; Rao & Knaus, 2008).

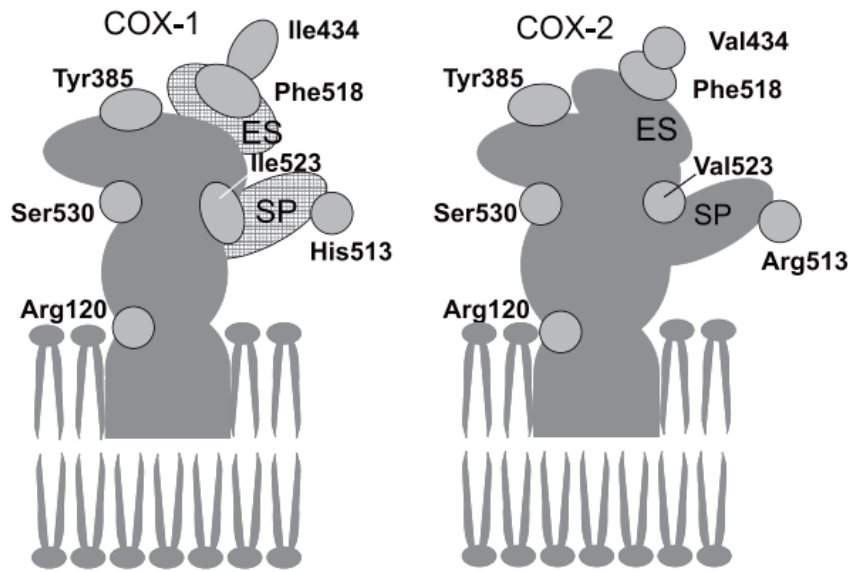


Figure 2: The structure of the COX-1 and COX-2 isoforms. The catalytic sites for the binding of NSAIDs include Tyrosine - TYR385, Serine - SER530 and Arginine - ARG120. Binding at these sites prevent the transforms of arachidonic acid to PGG₂. Note the other amino acid residues are Isoleucine – ILE, Valine – Val, Histidine – His, Phenylalanine – Phe. The structure of each enzyme creates a side pocket - SP and an extra space - ES. (Botting, 2010).

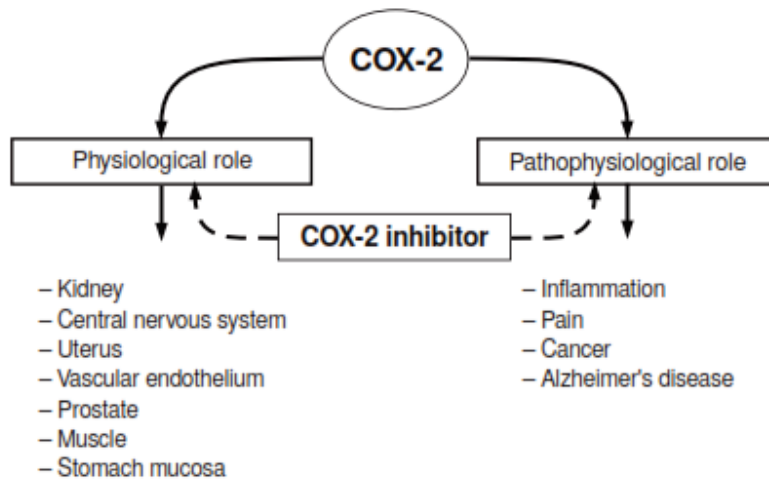


Figure 3: The physiological and pathophysiological roles that the COX-2 isoform has within the body (Steinmeyer, 2000)

1.2.3. Side effects of NSAIDs

Most of the NSAIDs' anti-nociceptive effects are exerted upon the spinal and supraspinal levels, which may account for the observed improvement in the overall well-being and increased appetite in human patients who have been treated with NSAIDs (Mathews, 2002). The choice of NSAIDs must be carefully chosen due to the fact that some drugs like aspirin, ketoprofen, and ketorolac are inhibitors of both COX-1 and COX-2, whereas drugs like meloxicam, carprofen and tolfenamic acid predominantly inhibit COX-2, but the inhibition of COX-1 is extremely small in comparison (Mathews, 2002).

For instance, humans should only be administered NSAIDs if they are 6 weeks or older, have normal renal and hepatic function, normal hemostatic function and no evidence or predisposition to gastric ulcerations (Trescot, Datta, Lee, & Hansen, 2008). Studies done on the effects of NSAIDs on the kidney show that COX-2 is an important enzyme that contributes to nephron maturation. If COX-2 inhibitor NSAIDs were to be given to 3 week old or younger canine pups, there may be permanent nephropathy. COX-2 selective inhibitors also show proteinuria and inhibition of glomerular sclerosis development in rats, resulting in a reduced functional renal mass. The conclusion from these studies suggested that if there are signs of dysfunction or abnormalities in these areas, the dose rate should be reduced to the minimal possible dose that retains the analgesic effect, or to sort out alternatives such as opioid therapy (Mathews, 2002). Although NSAIDs are used in both humans and animals, neither the dose nor safety of one drug in humans should be assumed

to be the same in animals, and vice versa, due to the narrow safety margins of each drug (Steinmeyer, 2000).

1.3. Use of NSAIDs in birds

The need for more information on adequate pain control in a wider variety of animals, including birds, is essential for improving their welfare. Mammals and birds have similar neural structures including neural pathways and neurotransmitters, however, the efficacy of NSAIDs between these two groups varies considerably (Desmarchelier, Troncy, Fitzgerald, & Lair, 2012). In general NSAIDs have a shorter half-life and metabolise faster in birds. But on the other hand, the clearance of the drugs takes a lot longer in birds when compared to mammals. Therefore, it is extremely hard to extrapolate the pharmacokinetic data between these two groups (Machin, 2005a).

Gathering more information about the physiological and pharmacology properties of NSAIDs in birds will help to eliminate the need to use extrapolated data. The inhibition on the production of arachidonic acid and thromboxane B₂ (TBX) due to NSAIDs can be used to determine the analgesic properties of various NSAIDs (Machin, 2005a). For instance, in mallard ducks (*Anas platyrhynchos*), both flunixin meglumine (5 mg/kg) and ketoprofen (5 mg/kg) suppress TBX levels for up to 12 h, which suggests that their physiological action may also last that long (Machin, Tellier, Lair, & Livingston, 2001).

Both mammals and birds can experience acute sharp pain following any fracture.

Pain following a fracture is associated with hyperalgesia due to the nociceptors in the periosteum becoming depolarised and inflammation of the surrounding tissue, thus activates the afferent pain pathways within the PNS which is perceived in the CNS (Webster, 2004). It is through the use of opioids and NSAIDs that can be given to help control the pain from such injuries. The use of opioids is often limited to the preoperative period, due to their short-term effects, whereas the NSAIDs used during the post-operative period due to their analgesic effects tend to have a much longer duration, some up to 12 hours post-administration (Desmarchelier et al., 2012; Lierz & Korbel, 2012; Machin, 2005a; Machin et al., 2001).

Through the development of numerous NSAIDs, many have been used to treat pain in birds, these include: acetylsalicylic acid, carprofen, celecoxib, dimeythsulfoxide, meloxicam, piroxicam, ibuprofen, phenylbutazone, ketoprofen and flunixin meglumine. (Desmarchelier et al., 2012). Production chickens (*Gallus gallus domesticus*) undergo partial beak trimming, those that have been treated with a topical dose of phenylbutazone were able to maintain their feeding intake levels for up to 24 h after the procedure, compared to untreated birds. Food laced with carprofen (1 mg/kg) was preferred by lame chickens when they were given a choice between laced or unlaced food. The consumption of the carprofen-laced food increased with the severity of lameness (Danbury, Weeks, Chambers, Waterman-Pearson, & Kestin, 2000). Broilers with chronic lameness showed significant increases in speed and walking ability with the increased carprofen. Also significant analgesic effects were seen in chickens (*Gallus gallus domesticus*) that had sodium-induced

synovitis when carprofen (30 mg/kg), flunixin (3 mg/kg) or ketoprofen (12 mg/kg) was administered (Machin, 2005a).

The major concern with using NSAIDs is the possible side effects that are known to occur in the gastrointestinal tract, renal system and hemostatic processes (Lierz & Korbel, 2012). Prostaglandins, in particular PGE₂, regulate the vascular tone and blood flow within the kidneys, especially during periods of decreased blood volume or systemic blood pressure. The inhibition of PGE₂ can result in ischemia of the kidney, the development of glomerular lesions and decreased uric acid secretion. If these effects go unnoticed it could result in hyperuricemia and severe renal failure (Sinclair et al., 2012).

Diclofenac is a NSAID that is widely used throughout veterinary medicine. In mammals the side effects of this drug are low but in birds it seems to be exceptionally toxic. One of the major impacts of diclofenac on birds was seen throughout the Asian subcontinent (Naidoo, Wolter, Cuthbert, & Duncan, 2009). The population of three endemic *Gyps* vulture species: Cape Griffon Vulture (*Gyps coprotheres*), African White-backed Vulture (*G. africanus*) and Oriental White-backed Vulture (*G. bengalensis*), took a steep decline in numbers due to diclofenac toxicity. However, this was not due to the direct administration of diclofenac, instead it was from the accidental exposure to contaminated cattle carcasses that still had residual amounts of diclofenac in their system (Naidoo et al., 2009).

From several studies using failure to thrive Cape Griffon vultures (*G. coprotheres*), the degree of sensitivity to diclofenac was apparent. Naidoo and colleagues found that after a small dose of diclofenac (0.8 mg/kg) vultures began showing signs of depression, anorexia, dehydration due to reduced water intake and began to droop their necks. The degree of these signs increased until they became semi-comatose and the death of both vultures occurred within 48 hours of administration (Naidoo et al., 2009). The results from the necropsy showed wide-spread lesions in the air sacs, heart and liver due to urate deposits. In the liver there was physical damage to the nucleated erythrocytes and the adjacent hepatocytes, and the lumen of the liver was filled with spicule and globular urates. The appearance of the kidneys in both birds were swollen, pale and granular, as well as injury and necrosis of the renal structures (Naidoo et al., 2009). All of these results were similar to the results Swan and colleagues found in the Oriental White-back vultures (*G. bengalensis*) (Swan et al., 2006).

Flunixin meglumine seems to have more adverse effects in birds than any other NSAIDs. For example, after a high dose of flunixin (10 mg/kg), regurgitation and tenesmus showed a significant increase in budgerigars (*Melopsittacus undulates*). Flunixin also showed nephrotoxicity in northern bob white birds (*Colinus virginianus*), renal ischemia and necrosis in Siberian cranes (*Grus leucogeranus*), muscular necrosis at the site of injection in northern bob whites (*Colinus virginianus*) and mallard ducks (*Anas platyrhynchos*), and renal gout in many avian species after repetitive use (Klein, Charmatz, & Langenberg, 1994; Machin et al., 2001). A combination of bupivacaine and ketoprofen

during propofol anaesthesia increased the mortality rates in male spectacled eiders (*Somateria fischeri*) and king eiders (*Somateria spectabilis*), which was primarily due to ketoprofen-mediated renal damage (Machin et al., 2001).

Meloxicam is a common NSAID that is used for its analgesic properties but only a few studies have been conducted which investigate the efficacy and analgesic effects of meloxicam in avian species (Machin, 2005b). Post-operative administration of meloxicam (2 mg/kg) has been shown to be the most effective in treating orthopedic pain following an osteotomy of the left femur in pigeons (*Columba livia*) when compared to the lower doses (0.5 and 1 mg/kg) or the control group. The pigeons (*Columba livia*) given the highest dose of meloxicam showed the ability to weight bear on the affected limb better than any other group in this trial (Desmarchelier et al., 2012). Moreover, the efficacy of meloxicam given at 1 mg/kg significantly reduced lameness and improved the Hispaniolan Amazon parrot's (*Amazona ventralis*) rotational perch performance. Therefore, these signs show the alleviation of pain, compared with doses that are ≥ 0.5 mg/kg (Cole et al., 2009).

Meloxicam is available in tablets, oral suspension (OA) and injectable via intravenous (IV) or intramuscular (IM) routes. Various studies have used Hispaniolan Amazon parrots (*Amazona ventralis*) to investigate the effects of meloxicam via these different administration routes (Molter et al., 2013). From the study conducted by Molter and colleagues the target plasma concentration of meloxicam which is known to result in

analgesia in the Hispaniolan Amazon parrots (*Amazona ventralis*) was 3.5 µg/mL. After 1 mg/kg was administered, the parrots who were injected IV or IM had plasma concentrations above this target concentration, with 3.7±2.5 and 3.5±2.2 µg/mL respectively. Furthermore, at 6 h post OA the average peak concentration was 3.5±1.2 µg/mL (Molter et al., 2013). Comparing the pharmacokinetic data of the different administration routes, the birds that were given meloxicam orally, bioavailability of meloxicam was between 49-75% compared to the bioavailability of IM or IV which were 100%. Whereas, the elimination half-life of meloxicam was similar between all 3 routes; 15.9±4.4, 15.1±7.7 and 15.8±8.6 h in IV, IM and OA respectively (Guzman, 2014).

Unlike many NSAIDs, meloxicam shows little to no adverse effects within the body after short-term use. Dijkstra and colleagues used Hispaniolan Amazon parrots (*Amazona ventralis*) to evaluate these possible side effects from blood, serum, faecal and urine samples following the administration of 1.6 mg/kg of meloxicam for 14 days. One of the main markers of renal toxicity is N-acetyl-β-D-glucosaminidase, which was examined in both urine and serum samples. Moreover, in the urine samples they screened for casts, specific gravity, protein, glucose, pH and haemoglobin, and the fecal samples were screened for occult blood. The serum samples were screened for: N-acetyl-β-D-glucosaminidase, glutamate dehydrogenase, alanine transaminase, aspartate aminotransferase, total protein, albumin and cholesterol. Also measured was the whole blood clotting time. From all of the screening from the various sample types, there was no evidence to suggest that at this dosage meloxicam had any effect on the renal, gastrointestinal system or on

hemostatic processes (Dijkstra et al., 2015; Guzman, 2014). Japanese quail (*Coturnix japonica*) were given meloxicam (every 12 hours for 14 days), the post-treatment uric acid concentration was significantly higher compared to the untreated quail. However, the mean value of these treated quail (7.8 ng/mL) was still within the acceptable range of uric acid concentrations. Histopathological studies of the kidneys showed no significant lesions due to the effects of meloxicam. The only adverse effect that was found during this study was muscle necrosis at the injection site. In particular, findings showed that 85% of the treated birds had haemorrhage and myositis within the pectoral muscle (injection site) (Sinclair et al., 2012). The use of meloxicam is commonly used due to the ease of administration. Both oral and injectable formulations are available in the concentrations, which are suitable for small patients. However, for birds who have pre-existing renal disease or are dehydrated, it is recommended to avoid the use of any NSAIDs, including meloxicam, as this may be fatal due to the increased pressure on the kidneys (Sinclair et al., 2012).

Meloxicam seems to be considered safe when administered to birds, but the adverse effects have not been well documented at this point in time. It is thought to be safer than flunixin, diclofenac and ketoprofen, however, there are still huge discrepancies of meloxicam's efficacy in avian species (Machin, 2005b).

1.4. Meloxicam pharmacokinetics in birds

There have only been a handful of studies investigating the pharmacokinetics of meloxicam in birds which contributes to the limited understanding we currently have about the mechanisms behind the pharmacokinetics of meloxicam. In *Table 2*, the summary of these studies shows the huge variation between various avian species.

On average the dose of meloxicam administered to the various species was 0.5 mg/kg; two studies increased the dose to 1 and 2 mg/kg in the Hispaniolan Amazon parrots (*Amazona ventralis*) (Molter et al., 2013) and the African White-back vulture (*Gyps africanus*) (Naidoo et al., 2008) respectively. The volume of distribution ranged between 0.832 L/kg in the Red-tailed hawks (*Buteo jamaicensis*) (Lacasse, Gamble, & Boothe, 2013) to 0.058 L/kg in chickens (*Gallus gallus domesticus*) (Baert & de Backer, 2002b). The clearance of meloxicam ranged from 1.675 L/h/kg in the Red-tailed hawks (*Buteo jamaicensis*) (Lacasse et al., 2013) to 0.0013 L/h/kg in chickens (*Gallus gallus domesticus*) (Baert & de Backer, 2002b). The half-life of meloxicam was the largest in the Hispaniolan Amazon parrots (*Amazona ventralis*) at 15.9 hours (Molter et al., 2013), along with chickens (*Gallus gallus domesticus*) and pigeons (*Columba livia*), where the drug stayed in the system a lot longer compared to some of the larger species.

Even though meloxicam had quite a higher clearance rate and was distributed widely in the tissues, it had quite a short half-life in the Red-tailed hawks (*Buteo*

jamaicensis), 0.78 hours. In both studies conducted by (Baert & de Backer, 2002a, 2002b), the MRT of the drug in the chickens was the highest, 4.41 hours. Whereas birds like Red-tailed hawks (*Buteo jamaicensis*), ostriches (*Struthio camelus*) and Great-horned owls (*Bubo virginianus*) had a small MRT with the drug being detectable for 0.38, 0.41 and 0.74 hours respectively. Most of the birds had high AUC readings, which were between 104 (Molter et al., 2013) and 3.372 h.µg/mL (Lacasse et al., 2013). However, the AUC readings for ostriches (*Struthio camelus*) and the Red-tailed hawk (*Buteo jamaicensis*) were relatively low compared to the other birds, 0.73 and 0.531 h.µg/mL respectively.

The largest bird species, the ostrich (*Struthio camelus*) (19 ± 6 kg) (Baert & de Backer, 2002b), showed that meloxicam was absorbed and distributed quickly into the tissues (V_d), which is also supported by the small half-life, AUC and MRT readings as they were removed from the systemic system quickly. Therefore, the concentration of the drug from the plasma sampled declined quickly. However, meloxicam remained in the tissues for an extended amount of time, leading to the clearance rate being high. On the other hand, the second heaviest bird, the turkey (*Meleagris gallopavo*) (8 ± 1.7 kg), did not follow such a trend. In fact, they showed no significant results when compared to the smaller birds in the pharmacokinetic parameters that were analysed. Some of the smaller birds like the Great-horned owls (*Bubo virginianus*) (2.2 kg) and the Red-tailed hawks (*Buteo jamaicensis*) (1.4 kg) (Lacasse et al., 2013) showed similar results like the ostriches. The other small birds tended to oppose this trend, whereby the V_d was small, so therefore clearance and the elimination rate (K_{el}) were proportional. Furthermore, the drug tended to stay a lot longer

in the systemic circulation, which is evident in the MRT and AUC values. This supports the difficulty in determining the dosage and dose rate for birds, as there are more intricate species differences that alter the pharmacokinetics of any drug, not just meloxicam. So just assuming dosage and dose rate based on the individual weight of the bird could easily be fatal if not monitored.

There seems to be an inversely proportional relationship between the half-life and weight of a bird. At a dose of 0.5 mg/kg, meloxicam has a shorter elimination half-life in ostriches (*Struthio camelus*) than in ducks (*Anas platyrhynchos*), pigeons (*Columba livia*) and chickens (*Gallus gallus domesticus*). As a result, smaller birds require longer dosing intervals than bigger birds (Machin et al., 2001).

Table 2: The mean \pm SD of the pharmacokinetic parameters of meloxicam in various bird species in the literature.

| Author | Species | N | Weight (kg) | Dose (mg/kg) | AUC (ng/mL.h) | K_{el} (h^{-1}) | V_{ss} (mg/ng/mL) | $t_{1/2}$ (hours) | C_i (L/h/kg) | MRT (hours) |
|--------------------------|-----------------------------|----|-----------------|--------------|------------------|-----------------------|---------------------|-------------------|--------------------|-----------------|
| Baert & de Backer, 2002a | Chicken | 6 | 2.2 \pm 0.2 | 0.5 | 40.79 \pm 5.87 | 0.22 \pm 0.04 | 0.058 \pm 0.005 | 3.21 | 0.0013 \pm 0.002 | 4.41 \pm 0.84 |
| Baert & de Backer, 2002b | Broiler Chicken | 6 | 2.2 \pm 0.2 | 0.5 | 20.2 \pm 2.9 | 0.22 \pm 0.04 | 0.117 \pm 0.01 | 3.2 | 0.025 \pm 4 | 4.41 \pm 0.84 |
| Baert & de Backer, 2002a | Ostrich | 6 | 19 \pm 6 | 0.5 | 0.73 \pm 0.18 | 6.09 \pm 4.58 | 0.59 \pm 0.19 | 0.5 | 0.72 \pm 0.2 | 0.41 \pm 0.25 |
| Baert & de Backer, 2002a | Duck | 6 | 3 \pm 0.8 | 0.5 | 8.38 \pm 1.32 | 1.62 \pm 0.61 | 0.065 \pm 0.017 | 0.72 | 0.061 \pm 0.01 | 0.77 \pm 0.2 |
| Baert & de Backer, 2002a | Turkey | 6 | 8 \pm 1.7 | 0.5 | 9.40 \pm 1.6 | 1.45 \pm 0.79 | 0.079 \pm 0.015 | 0.99 | 0.055 \pm 0.011 | 1.47 \pm 0.27 |
| Baert & de Backer, 2002a | Pigeon | 6 | 0.45 \pm 0.02 | 0.5 | 18.35 \pm 9.84 | 0.29 \pm 0.10 | 0.14 \pm 0.10 | 2.40 | 0.039 \pm 0.03 | 3.89 \pm 1.49 |
| Molter et al., 2013 | Hispaniolan Amazon Parrots | 11 | 0.285 | 1 | 104 \pm 54 | - | 0.232 \pm 0.22 | 15.9 | 0.012 \pm 0.014 | - |
| Lacasse et al., 2013 | Red-tailed Hawk | 7 | 1.4 | 0.5 | 0.531 \pm 0.28 | - | 0.832 \pm 0.711 | 0.78 | 1.675 \pm 1.59 | 0.38 \pm 0.37 |
| Lacasse et al., 2013 | Great Horned Owl | 7 | 2.2 | 0.5 | 3.372 \pm 1.39 | - | 0.1376 \pm 0.063 | 0.49 | 0.154 \pm 0.082 | 0.74 \pm 0.28 |
| Naidoo et al., 2008 | African White-back vultures | 6 | - | 2 | 6.29 \pm 2.63 | 2.57 \pm 0.0025 | 0.15 | 0.33 | 0.056 \pm 0.035 | - |

Scientific names for the bird species: Chicken (*Gallus gallus domesticus*), Ostrich (*Struthio camelus*), Duck (*Anas platyrhynchos*), Turkey (*Meleagris gallopavo*), Pigeon (*Columba livia*), Hispaniolan Amazon parrots (*Amazona ventralis*), Red tailed hawks (*Buteo jamaicensis*), Great horned owls (*Bubo virginianus*) and African White-backed Vulture (*Gyps africanus*).

1.5. Allometric scaling

As mentioned in section 1.3 and 1.4, the lack of research of NSAID pharmacokinetic information in avian species requires the extrapolation of data from mammalian species (Machin, 2005a). This ability to extrapolate data from one species to another is due to allometric scaling. Allometric scaling is based on the knowledge that many physiological functions and organ sizes follow an allometric relationship. Fundamentally it is based on the body mass or size of any particular species which share similar anatomical, physiological and biochemical characteristics and their Basal Metabolic Rate (BMR). Therefore, data from numerous species can be used to extrapolate information in order to construct a predicted linear relationship between animal species, and between animals and humans (Huang & Riviere, 2014). Interspecies allometric scaling can be expressed mathematically as:

$$Y = a \times W^b$$

Where Y is the parameter of interest,

W is the average weight of the species,

a and b represent allometric coefficients.

However, in order to use a and b in a linear regression, the equation above needs to be transformed through logarithms, which is expressed as:

$$\text{Log } Y = \log a + b \log W$$

This change allows for the relationship between Y and W to be better understood,

as $\log a$ and b become the y-intercept and slope respectively, creating a linear relationship (Huang & Riviere, 2014). Predicting the pharmacokinetic parameters across species requires the correlation with body weight. Therefore, it is essential to include animals that show great diversity in weight (0.1 - 5000 kg). By having at least 3 orders of magnitude of weight, a clearer linear relationship can emerge, generating extrapolated data for various other species. Advantages of using interspecies allometric scaling to predict pharmacokinetic parameters include: its simplicity; the pharmacokinetic parameters are calculated from the concentration-time curve data, which require the plasma concentrations; the difference in elimination pathways and plasma protein binding properties is helpful to know, but not essential; and the data analysis time is short (Mahmood, 2007).

In the absence of species-specific pharmacokinetic data, the process of allometric scaling is a beneficial approach to obtaining data (Hunter, Mahmood, & Martinez, 2008). Pharmacokinetic research into numerous pharmaceutical drugs has been conducted in common domestic species like dogs (*Canis lupus familiaris*), cats (*Felis catus*), cattle (*Bos taurus*) and sheep (*Ovis aries*), and laboratory animals like rats (*Rattus*), mice (*Mus*) and rabbits (*Oryctolagus cuniculus*). The same cannot be said for non-domestic species like birds, reptiles and fish (Hunter & Isaza, 2002). Due to the lack of pharmacokinetic data in the literature for species that are classified as exotic or wild, there is a lot of concern from clinicians who commonly deal with these types of species. The clinicians must extrapolate pharmacokinetic data from common mammals so that they can approximate dosage for these species (Hunter & Isaza, 2002).

The ability to extrapolate pharmaceutical data presumes that the animals share similar anatomical, physiological and biochemical characteristics regardless of the change in body mass. Therefore, it is only the dose of a drug which changes in response to differences in weight. However, even within mammalian species there is a broad range in the anatomical, physiological and biochemical characteristics. For example, the differences in the gastrointestinal tracts of mammals change when and where drugs are absorbed through the lumen walls (Hunter & Isaza, 2002). So if there are intra-species differences other than their body mass which need to be taken into consideration, there is a lot more needing to be taken into consideration when extrapolating data for animals like birds who differ completely in their anatomy, physiology and biochemical characteristics (Hunter & Isaza, 2002).

Drug metabolism and excretion are crucial in analysing the pharmacokinetics. Metabolism of a drug is an important function within the body in order to deactivate the drug and in some cases re-activated (Hunter & Isaza, 2002). This process occurs mainly in the liver where the drug undergoes several phase reactions. Phase I reactions use the cytochrome P450 enzymes to alter the structure of the drug allowing it to be further broken down. This set of enzymes has been found to vary between species, as well as within species. This ultimately will affect the dosage and dose rate, therefore will impact the data which is used for extrapolation (Hunter & Isaza, 2002). Clearance and elimination from the body primarily occurs in the kidneys, liver and feces. Therefore, it is important to consider

the anatomical and physiological differences that are seen between mammals and avian species. For example, the avian kidney does not possess the loop of Henle of the proximal tubules due to all the renal tubules being straight, unlike mammals. Furthermore, glomerular filtration (GFR) occurs intermittently in avian species due to their reproductive system, whereas in mammals filtration of the blood is a continuous process (Hunter & Isaza, 2002). See section 2.1.3 and 2.1.4 for more in depth explanations of drug metabolism and excretion.

The amount of knowledge about the mechanisms and use of Meloxicam in birds is extremely limited. Presently treating birds with meloxicam requires data from mammalian species to be extrapolated but such a method has potential complications for the livelihood of the birds. Furthermore, the studies that have been conducted in birds use species that are not commonly kept as companion animals, for instance, chickens (*Gallus gallus domesticus*) (Baert & de Backer, 2002a), quail (*Coturnix japonica*) (Sinclair et al., 2012), ostriches (*Struthio camelus*), pigeons (*Columba livia*) and ducks (*Anas platyrhynchos*) (Baert & de Backer, 2002a). The results from these studies show that there is a wide species differentiation between the effects of meloxicam. This limits the ability to extrapolate data between avian species especially when trying to deal with companion animals (Wilson et al., 2004).

Presently the traditional methods of allometric scaling have not been carefully investigated within avian species, therefore, veterinarians are apprehensive about using this technique. To gain more understanding surrounding this topic, Hunter and colleagues

explored if the application of allometric scaling upon avian species would alter the systemic clearance of drugs, depending on whether the data extrapolated was from mammalian data or from avian data (Hunter et al., 2008). Two methods were used in order to produce the allometric equations for this study. The first method used mammalian pharmacokinetic data from at least three mammalian species to predict the systemic clearance rate in birds. The second method, the pharmacokinetic data only came from avian species. These methods produced specific allometric equations which produced a predicted value for clearance, which was then compared to the observed values in several avian species. The resulting percent error from this two values shows how effective the extrapolation was (Hunter & Isaza, 2002). The percent error is calculated by using this equation:

$$\% \text{ error} = \frac{(\text{observed} - \text{predicted}) \times 100}{\text{observed}}$$

Overall the results showed that there was a smaller percentage error between the observed and predicted systemic clearance of drugs when only avian data was used, especially in the drugs that are primarily cleared in the renal system. However, using the same method for meloxicam showed a larger percentage error compared to the first method's results. Hunter and colleagues concluded this result was partly due to meloxicam being primarily cleared in the liver. Unlike the differences in the renal system between birds and mammals, their hepatic systems are relatively similar, especially in the hepatic vascularity. But it is the lowered overall cardiac output to the liver which reduces the amount of meloxicam being metabolised per minute compared to mammals like dogs (*Canis lupus familiaris*) or rats (*Rattus*). Furthermore, the p-glycoprotein mediated transport in

mammals is shown to differ from avian species (Hunter et al., 2008). This also need to be taken into account when analysing drugs that use the liver to primarily clear drugs in these species. The overall conclusion drawn from this study was that both methods produced percentage errors, which were species-specific but also drug-specific. Therefore, even if allometric scaling was done by avian data alone there would still be a risk associated with using extrapolated data, in addition, other variables like the specific drug and its primary site of clearance must be taken into consideration to ensure the upmost safety for the animal is upheld (Hunter & Isaza, 2008; Hunter et al., 2008) .

Conclusion

In conclusion, it is clear from this section that there are huge gaps in the literature regarding the use of NSAIDs in general but also for particular NSAIDs in avian species. This makes it harder to know how specific drugs interact within the body, the dose at which they have an analgesic effect and the side effects and potency of NSAIDs. All of which are shown to vary between avian species. Overall such a lack of knowledge makes it harder to treat and reduce pain in birds. If there was more literature in this area, the ability to extrapolate data from birds for birds would help to reduce the error associated with extrapolating from mammalian data and more importantly improve the safety of these drugs when administered to birds.

In the next section, the area of pharmacokinetics will be explored further to provide an understanding of the principles and mechanisms behind how substances differ in their pharmacokinetic properties as we have seen in this section.

2. Pharmacokinetics

The consistent use of NSAIDs for analgesia dates back as early as 1879, when aspirin was discovered (Vane & Botting, 2003). Over the decades, NSAIDs have diversified and become a popular pain relief method for both humans and animals alike. As a result, quantitative methods have been developed in determining the efficacy and safety of a substance. By studying the pharmacokinetics and pharmacodynamics of NSAIDs invaluable information has been provided on how the substances interact within the body and their cumulative effects (Abdel-Rahman & Kauffman, 2004). The concept of pharmacokinetics predicts the spatial and temporal distributions throughout the body from examining the time dependent concentration of a substance (Foster, 2007). This section focuses on the four principles of pharmacokinetics and the parameters from which pharmacokinetics are calculated. In addition, the different pharmacokinetic models are examined and compared in order to illustrate the different ways the parameters can be calculated when the body is divided up into theoretical compartments or non-compartments.

2.1 Pharmacokinetic principles

In pharmacokinetics the distribution of a drug within the body can be analysed through four key principles: absorption, distribution, metabolism, and elimination (*Figure 4*). Their associated parameters rely on different rate constants in order for the drug to move within the body until it has been completely eliminated or undetectable in the body. In zero-order reactions the concentration of the drug present in the body declines in a linear

fashion and the elimination rate of the drug is constant (Riviere, 2011b). For example if drug A (dA) is decreasing at a constant rate, the rate of elimination from the body can be illustrated by:

Where k^* is the zero-order rate constant.

In first-order reactions, the rate of a drug that is being eliminated is dependent on the concentration of the drug present in the body at the time. In order to create a linear model of elimination, it requires the data to be plotted on a semi-logarithmic axis (Riviere, 2011a). It can be illustrated simply by:

$$-kA = \frac{dA}{dt}$$

Where kA is the first-order rate constant.

Understanding and calculating the pharmacokinetic parameters for most drugs obey the first-order reactions (Riviere, 2011b).

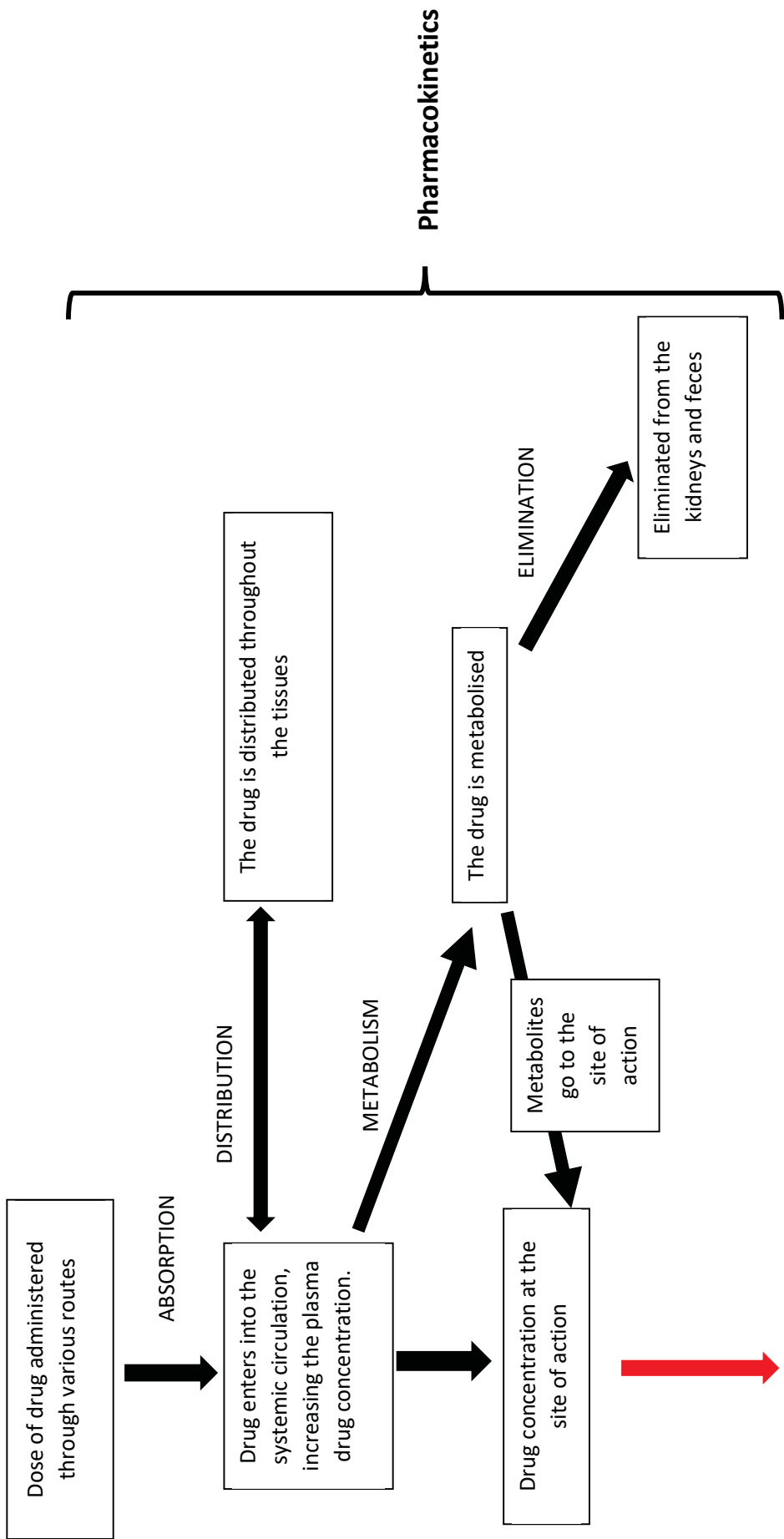


Figure 4: The relationship of the four pharmacokinetic principles, which influence the effect within the body (pharmacodynamics) (Katzung, Masters, & Trevor, 2009).

2.1.1. Absorption

The absorption of any drug is classified by its movement from the site of administration into the systemic circulation through passive or active transport. Drugs that are lipid soluble or non-ionised are able to passively enter the cell through the cell membrane diffusely down its concentration gradient. Active transport requires the presence of transmembrane proteins that are specific transporters with the cell membrane, which have specific selectivity towards structure and saturation, all of which requires energy, such as ATP, to transport these molecules into the cell. (Boothe, 2000; Riviere & Papich, 2009).

There are numerous ways that drugs can be introduced into the body which include oral administration (OA), intramuscular (IM), subcutaneous (SC), intraperitoneal (IP), intravenous (IV), buccal, rectal, vaginal, sublingual, inhalation and intrathecal (Riviere & Papich, 2009).

Orally

For drugs that are administered by tablet or capsule form, absorption into the systemic system requires the passage through the mucosal linings of the GI tract. Majority of drugs primary site of absorption is in the small intestine due to the alkaline pH, which reduces the harsh environments to which the drugs are exposed, and the small intestine is already conducive to absorption of many other particles. Orally administered drugs often

undergo what is known as first-pass metabolism. This is where drugs that are absorbed through the GI tract are absorbed into the portal circulation by enterocytes, and removed from the blood by hepatocytes in the liver (Riviere & Papich, 2009; Roberts, Magnusson, Burczynski, & Weiss, 2002).

Intramuscular/subcutaneous

This route of administration allows drugs to be injected into tissues that are well perfused with systemic capillaries which drain into the venous circulation. Regions that are often selected in both humans and animals are various muscle groups like the biceps, gluteal muscles and the quadriceps (flanks). Drugs that are known to be irritants should not be administered this way due to the increased pain in the SC areas as the drug diffuses (Riviere & Papich, 2009).

Intravenous

The administration of drugs using this method is instantaneous and requires zero absorption due to the drugs being directly injected into the systemic system. As a result, the bioavailability of the drugs is 100%. This enables the maximum concentration of the drugs to be distributed throughout the body and to assert their effects. However, they do require more invasive procedures compared to other routes of administration (Boothe, 2000; Katzung et al., 2009; Riviere & Papich, 2009).

2.1.2. Distribution

Distribution is crucial in maintaining the biological response of a drug. This is done by maintaining the concentration levels high enough in particular tissues or action sites for a sufficient amount of time. The distribution of a drug depends on various factors; pK_a , solubility, molecular weight, perfusion rate to the tissue/s and affinity for the plasma proteins. The only limitation of the distribution of the drugs, and consequently the volume of distribution, is that without specific biopsies of the specific tissues it is hard to determine where the drug has actually been distributed to in the body and at what concentration (Boothe, 2000; Riviere & Papich, 2009).

2.1.3. Metabolism/biotransformation

The role of drug metabolism in the body is to alter the chemical structure of the drug so that the correct receptor site is targeted, the drug-receptor affinity is maximised so the pharmacological effects are expressed and then excreted from the body. The metabolism of the parent drug results in the metabolite being deactivated, and therefore unable to interact with a specific receptor target site, or it is activated so that the pharmacological effects can be expressed. The intensity and the drug-associated toxicity of any drug is proportional to its concentration in the plasma and/or the active metabolites effect at the target sites (Riviere & Papich, 2009).

The liver is the major organ for drug metabolism, as well as being partly responsible

for drug excretion. The localisation and biotransformation of a drug depends on many factors, which include both the biological system of the liver itself, and the properties of the specific drug that has been introduced (Riviere & Papich, 2009). This process of drug metabolism requires various metabolic pathways, which can be divided up into phase I reactions and phase II reactions (Riviere & Papich, 2009). Phase I reactions convert the parent drug to a more polar metabolite by introducing a functional group (-OH, -NH², or -SH). If the addition of one of these functional groups make the metabolite polar high enough it will be excreted. If not, the metabolite will go on to phase II reactions (Katzung et al., 2009; Riviere & Papich, 2009).

Most phase I metabolites require further transformation to become more polar and water soluble so that they can be excreted. This occurs by conjugation, which is classified as phase II reactions (Riviere & Papich, 2009). Phase II reactions require energy and specific transfer enzymes, transferases. The most dominant enzymes in this process are the uridine 5'-diphosphate [UDP]-glucuronosyl transferases (UDTs) (Katzung et al., 2009). To further the metabolite's transformation various processes can occur, which include: glucoronidation, sulphate conjugation, glycine conjugation, acetylation and glutathione conjugation. These reactions occur a lot faster compared to phase I reactions (Katzung et al., 2009).

Meloxicam is metabolised extensively in the liver which produces four inactive metabolites (*Figure 5*). Phase I reaction produces the intermediate metabolite 5'-

hydroxymethyl meloxicam (AF-UH 1 SE), which is further metabolised by phase II reactions into a carboxylic acid metabolite (UH-AC 110 SE) and 2 oxoacetic metabolites (DS-AC 2 SE and BI-BO 8032 NA) (Davies & Skjodt, 1999).

2.1.4. Excretion/elimination

For most drugs after being metabolised in the liver, they are primarily eliminated through the renal system. Absorption of a drug is defined as the movement from outside the body into the systemic circulation, and elimination is the reversal of this process (Riviere & Papich, 2009).

The process of drug elimination in the kidney occurs in three distinct processes: 1) glomerular filtration (GFR); 2) active tubular secretion and/or reabsorption; and 3) passive flow-dependent non-ionic back diffusion (*Figure 6*) (Riviere & Papich, 2009). GFR is non-directional, which filters out the larger molecules like erythrocytes, white blood cells and plasma proteins. Drugs that are unbound from the plasma proteins undergo tubular secretion which requires the assistance of the active $\text{Na}^+\text{-K}^+$ ATPase transport system and energy in the form of ATP to move and transport the drug molecules in or out of the tubules (Riviere & Papich, 2009).

In the proximal tubules, reabsorption is through passive transport. As the water is being reabsorbed it creates a large concentration gradient between the drug in the distal tubule and the freely unbound drug in the blood (*Figure 7*) (Baggot, 2001). As a result, the

drugs are excreted out of the body through the production of urine. Drugs that have a high lipid solubility, molecular weight and polarity tend to have a high binding affinity to plasma proteins that cannot be filtered through the kidneys. Therefore, the bile is used as a medium to remove the drugs from the body via faeces. In some cases the bile disassociates the drug from the plasma proteins which can then be reabsorbed from the blood into the tubular system of the kidneys (Baggot, 2001; Riviere & Papich, 2009).

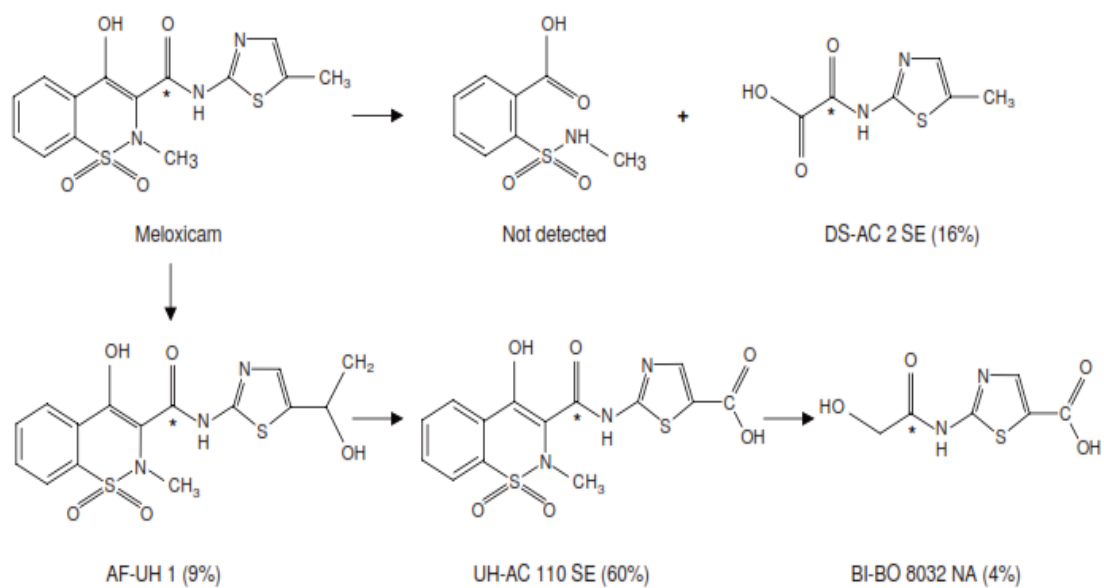


Figure 5: The metabolism of meloxicam into the four metabolites. The main metabolism pathway of meloxicam produces an intermediate metabolite 5' hydromethyl meloxicam (AF-UH 1), which is converted to a carboxylic acid metabolite (UH-AC 100 SE) then an oxoacetic metabolite (BI-BO 8032 NA). A smaller pathway converts meloxicam into another oxoacetic metabolite (DS-AC 2 SE) (Davies & Skjold, 1999).

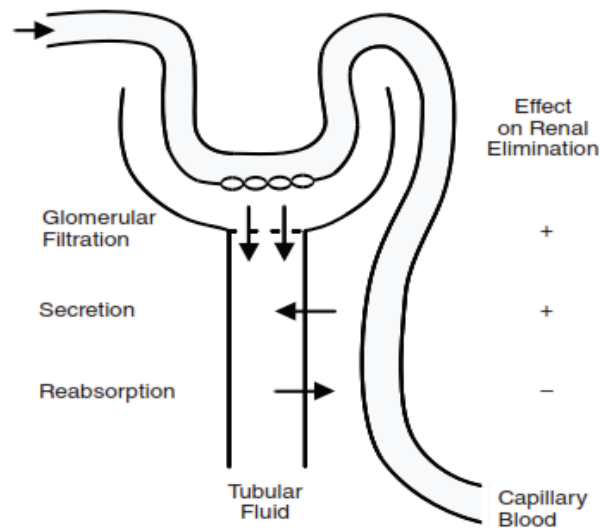


Figure 6: The three stages which aid in the overall rate of drug eliminated from the renal system (Riviere & Papich, 2009).

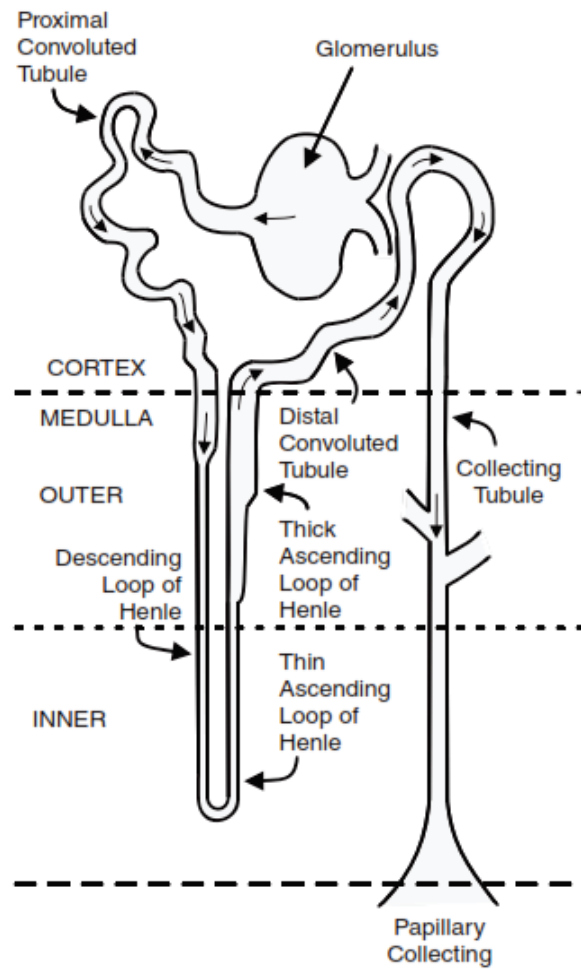


Figure 7: The anatomical and physiological processes within the mammalian nephron which aid in drug excretion and urine production (Riviere & Papich, 2009).

2.2. Pharmacokinetic parameters

Pharmacokinetic parameters are derived from pharmacokinetic models which describe the kinetics of a drug through the body in relation to the four pharmacokinetic principles. The calculation of these core parameters ensures that experiments are based on common concepts which are transferrable to other studies investigating the efficacy of a drug and the comparisons of other drugs and their efficacy. They also aid in the development of new or existing drugs, as they allow scientists to determine their pharmacological actions including their pharmacodynamic actions within the body (Foster, 2007; Jang, Harris, & Lau, 2001).

2.2.1. Area under the curve (AUC)

The area under the concentration time curve represents the total amount of the drug present in the plasma over a period of time. It is dependent on how much of the drug enters the systemic circulation and the body's ability to eliminate the drug (Riviere, 2009a). The AUC can be used to calculate the bioavailability of a drug, if the AUC is calculated from time 0 (administration) to infinity. AUC readings are expressed as mass x time/volume (ng·h/mL).

The most accurate method to obtain the AUC is by applying the trapezoid rule (*Figure 8*) or integration. Both the AUC (zero moment) and the AUMC (first moment) curve are calculated from time 0 to the last plasma drug concentration and extrapolation to infinity time is also incorporated (Riviere & Papich, 2009).

AUC is calculated by:

$$AUC = \frac{C_{(last)}}{\beta}$$

AUMC is calculated by:

$$AUMC = \frac{t * C_{(last)}}{\beta} + \frac{C_{(last)}}{\beta}$$

Where β is the overall elimination rate constant

t^* is the time of the last measured plasma drug concentration ($C_{(last)}$)

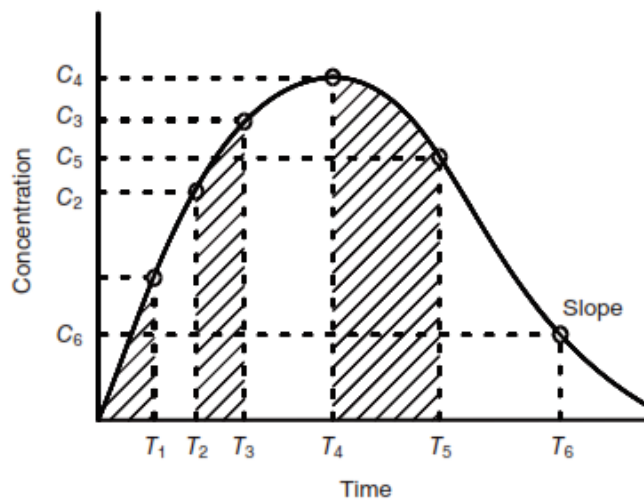


Figure 8: The plasma concentration – time curve broken down into trapezoids which is used to calculate the AUC and AUMC (Riviere & Papich, 2009).

2.2.2. Bioavailability (F)

Bioavailability is defined by the absolute concentration of the drug in the systemic circulation after the administration of the drug. There are various ways in which drugs can be administered so the time taken to reach the systemic circulation can vary significantly. Orally administered drugs take the longest time to enter the systemic circulation, which is reflected by a decreased bioavailability, in some oral drugs only 50% of the drug reaches the circulation. In contrast, the direct administration of drug via intravenous injection results in the bioavailability of 100% (Riviere & Papich, 2009).

$$F = \frac{AUC \text{ route} \times Dose \text{ IV}}{AUC \times Dose \text{ route}}$$

2.2.3. Volume of distribution (V_d)

This term describes the theoretical volume of fluid that is needed for the concentration of the distributed drug in the body to be the same concentration that is in the plasma (Katzung et al., 2009). Drugs that are widely distributed and have a high V_d tend to have a higher lipid solubility and a lower affinity to plasma proteins, as a result, they are removed from the circulation quickly. In comparison, drugs that have a low V_d have a lower lipid solubility and have a higher affinity to the plasma proteins which results in the drug remaining in the circulation a lot longer (Baggot, 2001; Riviere & Papich, 2009). The equation for V_d is illustrated below and is expressed as mL/kg.

$$V_d = \frac{Dose}{C_{max}}$$

2.2.4. Clearance (Cl)

Clearance is the volume of plasma which has been irreversibly cleared of a drug per unit of time. This parameter examines the sum clearance of the drug from the various organs of elimination. The total amount of volume cleared per unit time is independent of the plasma drug concentrations so the clearance is universal in all of the organs. However, if the plasma concentrations are so high that organs like the liver become saturated it may take longer to fully clear the drug (Boothe, 2000).

There are various ways to calculate the clearance of a particular drug which include:

$$Cl = \frac{F \times Dose}{AUC}$$

Where F is Bioavailability.

If the drug is administered via IV, where F = 100%, the equation would be:

$$Cl = \frac{Dose}{AUC}$$

The clearance of a drug can also be calculated through knowing the V_d and elimination rate constant K_{el} (or half-life), as clearance of a drug is represented by the fraction (K_{el}) of the V_d of a drug per unit of time (Boothe, 2000), which is expressed as:

$$Cl = Vd \times Kel$$

Where the elimination rate constant is determined by the fraction of drug eliminated per unit time and it is the slope of the log concentration/time curve:

$$K_{el} = \frac{\ln C_1 - \ln C_2}{t_2 - t_1}$$

2.2.5 Half-life ($t_{1/2}$)

Pharmacokinetically, the half-life of a drug is a 'pure' parameter as it can be directly measured from the plasma drug concentration-time curve (*Figure 9*). Physiologically, the half-life of a drug is classified as a 'hybrid' parameter because it is impacted by both the distribution and the clearance. Evidently, if there is a wide distribution of the drug throughout the body, the time taken to clear it from those organs may take longer, therefore increasing the half-life. Furthermore, if the organs of clearance cannot access the drug to successfully remove it, the rate of elimination decreases and the elimination half-life of the drug changes in direct proportionality with the volume of distribution which becomes inversely proportional with the clearance of the drug. This response is typically seen in patients who are dehydrated or have chronic renal disease (Boothe, 2000).

Due to the huge species variation in the rate of elimination of a drug, the half-lives also vary between species. These differences are often related to the altered liver metabolism and renal excretion in a species. The quantitative measure of a drug's elimination rate is provided by analysing the amount of time taken for the plasma concentration to decrease by half (Riviere, 2009b). This information is useful when establishing the dosage intervals of any particular drug. Repeated administration of a drug

leads to the concentration within the plasma getting to a steady state, which causes the elimination rate of the drug to equal the rate of administration. By maintaining the plasma concentration at a therapeutic level, drugs like NSAIDs become very effective at controlling the pain an animal may experience (Godin, 1996).

The half-life is determined by the equations:

$$t_{\frac{1}{2}} = \frac{0.693 \times V_d}{C_l}$$

or

$$t_{\frac{1}{2}} = \frac{0.693}{k_{el}}$$

The value of 0.693 is the natural logarithm of 2

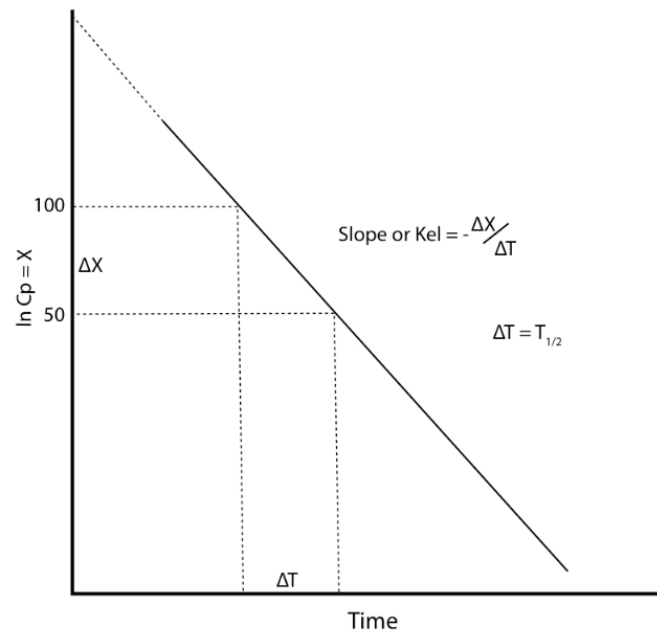


Figure 9: Graph showing the method for calculating the half-life on the concentration-time curve using the semi-log plot. The X-axis is the log of concentration (x) and the Y-axis is time (t) (Riviere & Papich, 2009).

2.2.6. Mean residence time (MRT)

After the administration of a single dose of a drug, the mean residence time (MRT) is the average time the molecules remain in the body (Baggot, 2001). Through calculating the AUC and AUMC from the plasma concentration time curves the MRT can be determined by:

$$MRT = \frac{AUMC}{AUC}$$

2.3. Compartmental pharmacokinetics

To analyse the pharmacokinetic aspects of any drug, several models can be used which view the body as if it was divided up into theoretical compartments. These compartments are not assigned specific body regions, so therefore it can be assumed that the rate of transfer between them is constant. Compartmental analysis relies on the linearity of the slope produced by the logarithmic concentration-time curve, therefore a drug must obey the first-order reaction rate (this was covered in section 2.1) (Riviere, 2009a). The most commonly used and easiest model is the one-compartmental open model (*Figure 10*). In this model, it is viewed that the body consists of one central compartment, where the movement of the drug occurs at a single rate throughout the body (Riviere, 2009b).

In this model, the rate of elimination per unit of time (K_{el}) of the drug in plasma is assumed to be the same for the other body fluids and tissues (Riviere, 2009b). The volume distribution is the volume of the compartment which the dose of a drug (D) instantaneously is distributed into. This means that the V_d is proportional to the dose administered and the concentration. It is calculated from extrapolating at $t=0$, which provides the concentration at time 0 (C_{p0}), and this is expressed as:

$$V_d = \frac{D}{C_{p0}}$$

This addition of V_d completes the equation for the one-compartment model, which is expressed as:

$$C_p = \left(\frac{X_0}{V_d}\right) x e^{-k_{el}t} = C_{p_0} x e^{-K_{el}t}$$

Where X represents the entire dose administered

and C_p is the plasma drug concentration at any time.

It can be simplified to:

$$C_p = C_{p_0} x e^{-K_{el}t}$$

The clearance of a drug occurs mainly in the kidneys by GFR, secretion, and absorption. In a one compartmental model clearance is defined in such a way that the kidneys are not specifically identified. Instead the body is considered one compartment, so it is defined as the body clearance (C_{lB}), which is expressed as:

$$C_{lB} = V_d x K_{el}$$

Although this model is conceptualised as one compartment, C_{lB} is the theoretical sum of the clearance from the hepatic system, renal system or any other organ which may be involved:

$$C_{lB} = C_{l_{Hepatic}} + C_{l_{Renal}} + C_{l_{Other}}$$

The last parameter is the half-life ($t_{1/2}$), which as mentioned in section 2.2.5 it is

the time taken for the concentration to drop by 50%. Calculating the half-life in this particular model is dependent on the volume of distribution and the clearance from the whole body, which is expressed as:

$$t_{\frac{1}{2}} = \frac{0.693 \times V_d}{C_{lB}}$$

Although the one compartment model is the simplest model which produces quantifiable results there are limitations to this model. Primarily it does not taken into consideration that a drug's movement and elimination are not uniform throughout the body (Riviere, 2009a). The two compartmental model (*Figure 11*) theorises that there are two compartments, the central and peripheral. The central compartment like the one compartment model, is where the drug is distributed and eliminated from. In addition this model takes into consideration that the drug distributes into other regions of the body (peripheral) at a different rate than that of the central compartment (Riviere, 2009b). The central compartment is viewed as the plasma and extracellular fluids from the highly perfused organs such as the heart, lungs, liver and kidneys. Whereas, the peripheral compartment is the plasma and extracellular fluid of the lesser perfused organs. This difference in compartments alters the distribution rate constant when a drug is distributed. The distribution from the central compartment to the peripheral compartment the distribution rate constant is annotated as K_{12} , whereas, when it is redistributed from the peripheral compartment back into the central compartment the distribution rate constant is annotated as K_{21} (Riviere, 2011b).

The equation of a two-compartmental model is (Figure 12):

$$C_p = A_1 \cdot e^{-\alpha t} + A_2 \cdot e^{-\beta t}$$

Where A_1 and A_2 are mathematical coefficients

α is the distribution rate constant

β is the elimination rate constant

As mentioned above, the drug is distributed from the central compartment into the peripheral compartment. Therefore, more calculations are needed to calculate the parameters of a drug like V_d and half-life. For example, to determine the V_d of a drug there are four calculations which are used, they include: volume of the central compartment (V_{dc}); volume of distribution at a steady state (V_{ss}); volume of the peripheral (V_{dp}); and the total volume of distribution in the body (V_{dt}) (Riviere, 2009a). They can be calculated by the following equations:

$$V_{dc} = \frac{Dose}{(A_1 + A_2)} = \frac{Dose}{C_{p_0}}$$

$$V_{ss} = \frac{V_{dc} \cdot [(k_{12} + k_{21})]}{[k_{21}]}$$

$$V_{dp} = V_{ss} - V_{dc}$$

$$V_{dt} = V_{dc} + V_{dp}$$

Referring back to the equation which calculates the two-compartmental model, the half-life must be calculated for both rate constants, α and β respectively. This is shown by the equations below:

The distribution half-life

$$t_{\frac{1}{2\alpha}} = \frac{0.693}{\alpha}$$

The elimination half-life

$$t_{\frac{1}{2\beta}} = \frac{0.693}{\beta}$$

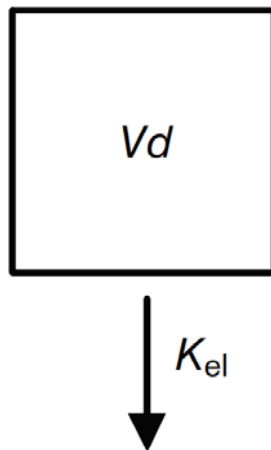


Figure 10: One-compartment pharmacokinetic model (Riviere, 2011b).

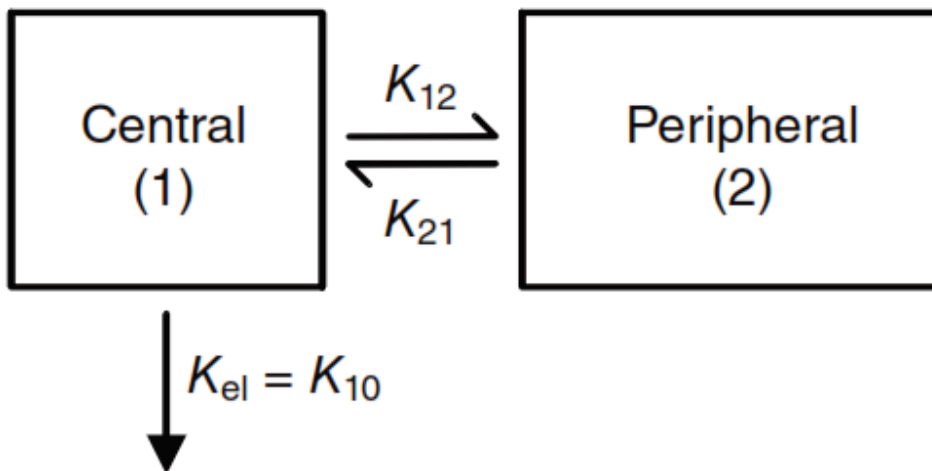


Figure 11: Two-compartmental pharmacokinetic model, which shows the distribution rate constants from the central compartment (k_{12}) and back to the central compartment (K_{21}) (Harrison & Lightfoot, 2006; Riviere, 2011b)

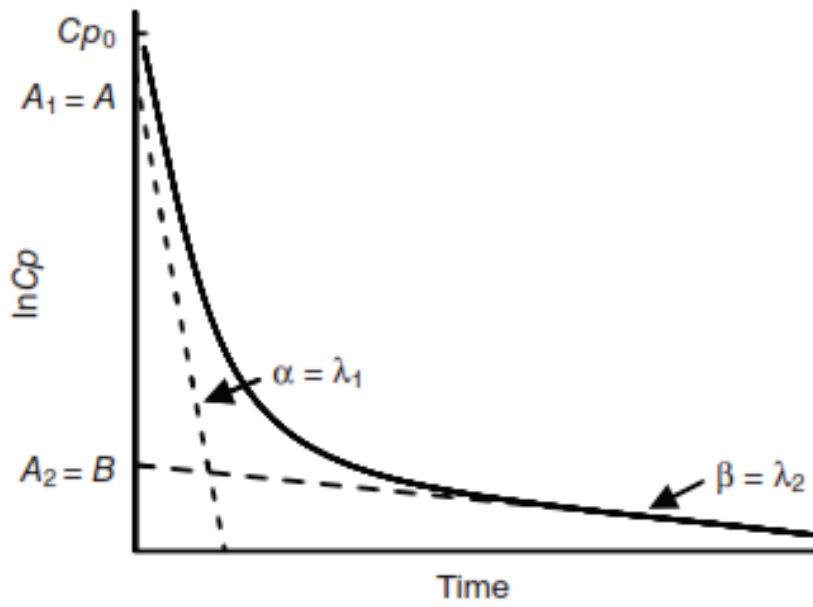


Figure 12: The two-compartmental model shown on the semi-log plot of the concentration-time curve (Riviere, 2011b).

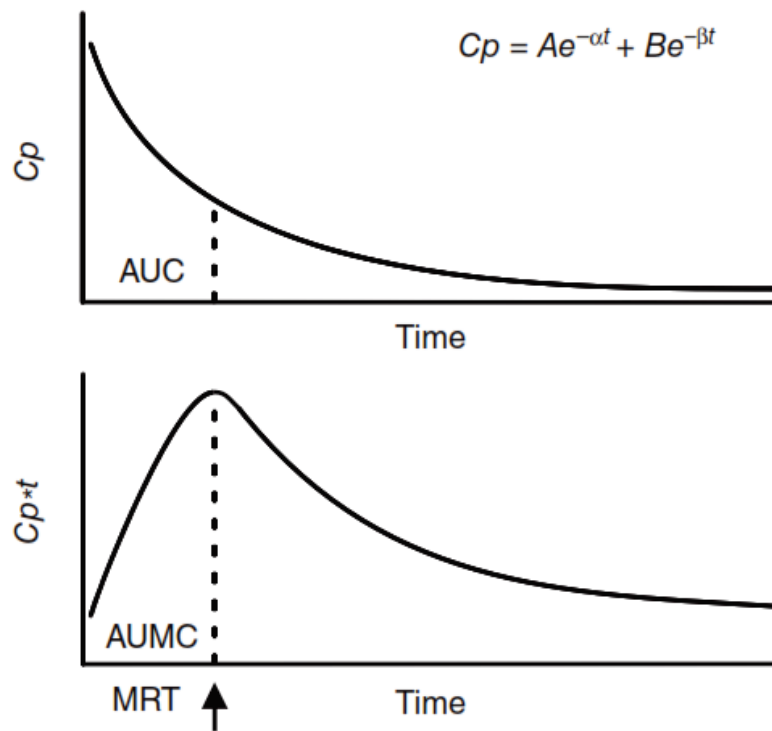


Figure 13: The concentration-time curve demonstrating that from the AUC and AUMC (broken lines) the MRT (the solid arrow) is calculated (Riviere, 2011b)

2.4. Non-compartmental pharmacokinetics

The use of non-compartmental models has been around for decades and was first implemented for the analysis of radiation decay. It was not until 1979 that this model was used to analyse pharmacokinetic problems (Riviere, 2011b). Like the compartmental models, the non-compartmental model is a theoretical model and is based on a section of the stochastic modelling approach called the statistical moment theory. However, it does not rely on the concept of theoretical compartments within a system (body) (Foster, 2007). This model examines the behaviour of the molecules within the body, more specifically, this model relies on the Mean Residence Time (MRT). In order to calculate the MRT, it requires the moments under the curve (AUC and AUMC) (*Figure 13,*) which are estimated from the concentration-time graph (Riviere, 2011b).

$$MRT = \frac{AUMC}{AUC}$$

*the calculations for both AUC and AUMC are shown in section 2.2.1.

This model can calculate other pharmacokinetic parameters using the AUC and MRT, which include the clearance (C_l), half-life ($t_{1/2}$) and the volume of distribution at a steady state (V_{ss}). There is significant difference between the theory behind the apparent volume distribution (V_d) (compartmental model) and the volume of distribution at a steady state (V_{ss}). Apparent V_d describes the theoretical volume of fluid that is needed for the concentration of the distributed drug in the body to be the same concentration that is in

the plasma (Katzung et al., 2009) which relies on the relationship between the concentration of the drug and the amount of drug that is in the body but does not necessarily correspond to the anatomical/physiological volume in the body (Riviere, 2011b). Whereas, the V_{ss} depicts the physiological properties of the drug instead of a theoretical volume. This parameter reflects actual blood and tissue volume in which the drug is distributed into and the relative binding of the drug to the protein in these spaces. The major factors that seem to have the greatest influence upon determining the V_{ss} are the blood protein binding and tissue binding properties of a drug. Unlike the apparent V_d , the elimination rate (K_{el}) of the drug does not seem to have a strong influence upon the V_{ss} (Riviere, 2011b). The equations for these parameters are shown below:

$$C_l = \frac{Dose}{AUC}$$

$$V_{ss} = C_l \times MRT$$

$$t_{\frac{1}{2}} = 0.693 \times MRT$$

Both of these pharmacokinetic models are useful in calculating the pharmacokinetic parameters, and in theory both models should produce the same answer. However, the small differences between the models often produce different results. When comparing these two models, there are advantages and disadvantages for both models. For instance, non-compartmental models only use linear events in order to calculate the parameters, while compartmental models use both non-linear and linear events. As a result, non-

compartmental models can only calculate the MRT, which is a basic overview of the drug's time within the body unlike the more in-depth information that compartmental models give especially in terms of the four pharmacokinetic principles (Foster, 2007). The advantage of using the MRT and AUC to calculate the other pharmacokinetic parameters is that it takes data straight from the plasma concentration-time curve, unlike the compartmental models.

Furthermore, the compartmental models have various assumptions that must be adhered to in order to calculate the various parameters. For instance, it is assumed that the clearance and elimination of a drug is constant throughout the compartments in order for these parameters to be calculated. However, fewer assumptions are required in non-compartmental models which reduces the errors that affect the final outcome (Foster, 2007; Riviere & Papich, 2009).

Conclusion

Understanding the area of pharmacokinetics is a complex and intricate process as there is huge variability in the interaction one particular substance has on the body and the complexity within the body itself when you consider the vast physiological functions and processes that occur when processing a substance. It is the four pharmacokinetic principles; absorption, distribution, metabolism and excretion, which give an outline of the processes the body goes through in order to utilise and then remove a certain substance. To examine the specific pharmacokinetic properties of a substance there are numerous

pharmacokinetic parameters (Section 2.2) which can be calculated from the plasma concentration-time curve which details a substance's movement in the body. It is evident that depending on what theoretical model is used and the assumptions of what state the body is considered to be in can alter the calculations of the pharmacokinetic parameters, resulting in varied pharmacokinetic properties of a substance.

It has already been discussed in section one that there is huge variation in the pharmacokinetics of NSAIDs between different species especially in avian species. The next section will explore a variable which has had little investigation in to the effect upon the pharmacokinetics of a substance, let alone drugs like NSAIDs. As an organism progresses in age, there are numerous age-related changes that can occur in the body. These changes often present as a decline in an organ/s function and is unable to keep up with the demands put upon it which can lead to disease (Harman, 1981). The next section will focus on the age-related changes that occur in organs associated with the processing of pharmacological drugs the age-related changes in avian species which effect the pharmacokinetics of NSAIDs

3. Age

Birds are becoming more popular as pets and more is being done in the conservation efforts to save exotic and endangered birds, as a result, they have longer life expectancy due to better veterinarian interventions preventing and treating diseases. Section one shows there is clear evidence that numerous NSAIDs are effective in treating pain in avian species, but an area that has not been investigated within avian species is whether the age has an effect upon their ability to process drugs like NSAIDs. This section aims to highlight the aging processes in avian species which have a direct impact on their ability to process NSAIDs, predominantly in the hepatic and renal systems. Additionally, this section will explain the pharmacological and pharmacokinetic changes that occur in humans as they age, in order to give an idea of the possibilities of age-related changes that could occur in birds and the way that their bodies process NSAIDs and the precautions needed when treating geriatric birds.

3.1. Aging birds

Aging patterns are organised into three different models which describe the various ways in which animals tend to age (Holmes, Fluckiger, & Austad, 2001). The first pattern depicts animals who have rapid senescence, but can suddenly die, for example anadromous salmon. The second and third patterns describe animals who have gradual senescence with a definite life span, or those that have a negligible senescence respectively. Homiothermic

vertebrates, which includes birds, fall into the second aging pattern which contributes to the extended life expectancies we see in these species. In general, birds tend to age a lot slower than mammals, but this is highly dependent on the species. Compared to mammals of similar body mass, most of the bird species have relatively long life expectancies and in some cases they live up to three times longer (Holmes, 2004; Holmes et al., 2001). For instance, the avian order Galliformes which includes the domestic chicken and coturnix quails, has a relatively short life span and ages the quickest. Compare this to the avian orders such as Psittaciformes (parrots), Charadriiformes (sea birds), Passeriformes (song birds) and Falconiformes (raptors), which all tend to have extremely long lives and show a very slow rate of aging relative to their body size (*Table 3*) (Holmes et al., 2001).

In the poultry production sector, it is unlikely that the birds reach the geriatric stage of life due to the fast turnaround needed to meet the supply demands. As a result, medical intervention is not required for these birds to extend their life. This is also true if a bird becomes injured or sick during the course of its lifetime. In comparison, intervention is sought to improve and extend the life of birds that are kept as pets or are endangered species living in zoos or sanctuaries. These birds are more likely to reach the age where they are considered to be geriatric. We know in humans, over the age of 65 is classified as geriatric (Hilmer, McLachlan, & Le Couteur, 2007), but there is no such age point at which we refer to birds as geriatric, which often poses a lot of issues when treating them. Often it is not until birds start showing signs of age-related health issues or a decline in health that they are classified as geriatric. As an example *figures 14, 15, 16* show the physical changes

of Grey Parrots (*Psittacus erithacus*) from their juvenile stage to adult stage, including an individual bird who is 45 years old which is considered to be extremely old for a bird (Doneley, 2011). Just like humans, there are numerous age-related issues that start to appear once they have reached 'old age'. Due to such an extended lifespan for some bird species, management of their health must take into consideration various comorbidities that can affect the treatment plans (Baine, 2012).

The role of the liver in metabolising drugs was described in section 2.1.3. The physiology of drug metabolism is very similar in a healthy bird (*Figure 17*) to that of a human's liver. One of the leading health concerns in older birds is chronic liver disease (Baine, 2012). This can occur because of various factors including injury to the liver overtime, chronic heart disease, malnutrition or chronic hepatitis. The liver is an organ that is quite resilient to stress and injury but over time pharmaceuticals, mycotoxins, heavy metals and bacterial or viral infections can cause irreversible damage to the liver which can lead to liver failure and/or cirrhosis which is often picked up once they are well into their 'old age' (*Figure 18*). The detection of chronic liver disease is often detected secondarily of primary issues which could involve the integumentary, gastrointestinal, renal, and respiratory systems (Baine, 2012).

In section 2.1.4 the physiological process of drug elimination and excretion within the renal system was discussed. In a healthy bird, the elimination of NSAIDs is relatively straight forward, the only difference is found in the anatomy of a bird's renal system

(*Figure 19*) compared to that of a mammalian's renal system (*Braun, 1998*). Due to the renal system being the main site of elimination of drugs (*Riviere & Papich, 2009*), with age, birds can develop chronic renal disease which can progress into renal failure (*Baine, 2012*). The most common causes of such a disease include dehydration and nutritional factors that include: hypovitaminosis A; dietary vitamin D3 toxicity; and excess dietary calcium; other toxicities from heavy metals, pharmaceuticals; bacterial nephritis; articular gout from increased uric acid; and neoplasia (*Lightfoot, 2010*). The clinical signs associated with chronic renal disease are non-specific but often include weakness, lethargy, anorexia, regurgitation, polyuria, polydipsia, lameness, and joint swelling. Management of this disease include the treatment of the underlying disease, diuresis through the administration of a balanced isotonic fluid and nutritional support to prevent any further damage (*Baine, 2012*).

In older birds with renal disease, pain management for symptoms like articular gout (*Figure 20*) should be done with caution, especially with the administration of NSAIDs. The stress upon the already compromised function of the kidneys can cause further damage, leading to complete renal failure. Whilst undergoing treatment for renal disease and the associated symptoms, if the analgesics which are required to ease the discomfort are ineffective in reducing the pain, euthanasia must be considered, especially if they are geriatric birds (*Lightfoot, 2010*).

Table 3: The life stages of various bird species (Doneley, 2011; Harrison & Lightfoot, 2006).

| Species | Weaning age (days) | Adult body weight (g) | Geriatric* (years) | Life expectancy (years) |
|---|-----------------------|--------------------------|-----------------------|----------------------------|
| Budgerigar (<i>Melopsittacus undulatus</i>) | 30-40 | 35-45 | 6-12 | 7-12 |
| Cockatiel (<i>Nymphicus hollandicus</i>) | 40-50 | 80-100 | 12-18 | 10-15 |
| Cockatoo (<i>Cacatua sulphurea</i>) | 80-90 | 310-380 | 20+ | 40-60 |
| Rainbow Lorikeet (<i>Trichoglossus oluccanus</i>) | 60-70 | 130-150 | - | 15-20 |
| Lovebirds (Agapornis roseicollis) | 45-55 | 40-50 | 10-15 | 7-15 |
| Indian Ring-neck parrot (<i>Psittacula krameri</i>) | 70-90 | 110-120 | - | 25-30 |
| Kiwi (<i>Apteryx haastii</i>) | 70-85 | 330 | - | 25-50 |
| NZ harrier hawk (<i>Circus approximans</i>) | 31-34 | 650-850 | - | 18 |
| Kakapo (<i>Strigops habroptilus</i>) | 27-31 | 1000-4000 | - | 50+ |
| Kea (<i>Nestor notabilis</i>) | 22-26 | 900-1100 | - | 22 |
| Kereru (<i>Hemiphaga novaeseelandiae</i>) | 27-30 | 630 | - | 21 |
| Morepork (<i>Ninox novaeseelandiae</i>) | 20-30 | 175 | - | 6 |
| Layer hens (<i>Gallus gallus domesticus</i>) | 112-126 | 1000-3000 | - | 2 |

*determination of the age at which these psittacines birds are classified as geriatric is based on anecdotal data from birds that have been bred in captivity over several generations.



Figure 14: A group of juvenile grey parrots (Doneley, 2011).



Figure 15: Adult Grey parrot (Doneley, 2011)



Figure 16: A 45 year old Grey parrot (Reavill & Dorrestein, 2010)

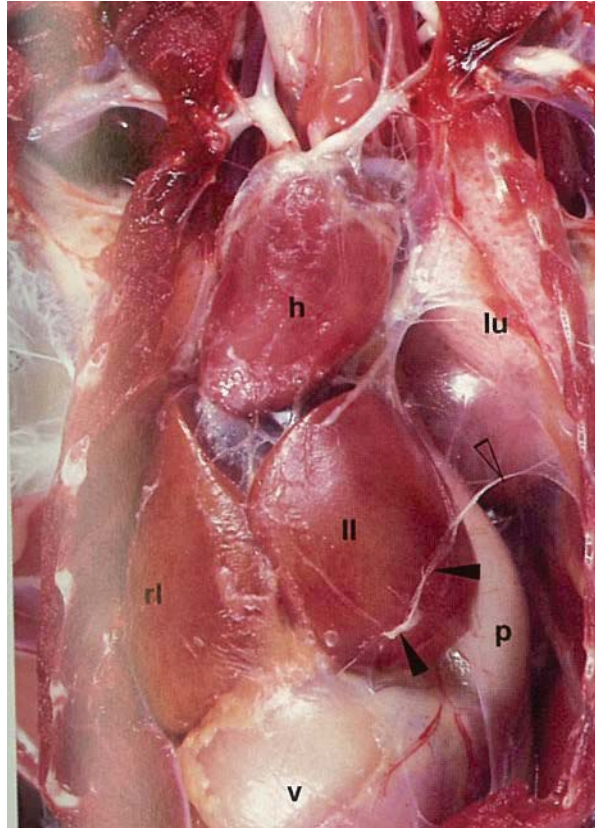


Figure 17: An avian (*Psittaciformes*) liver with (rl) right lobe of liver, (ll) left lobe of liver, (lu) lung, (h) heart, (p) proventriculus and (v) ventriculus (Ritchie et al., 1999).

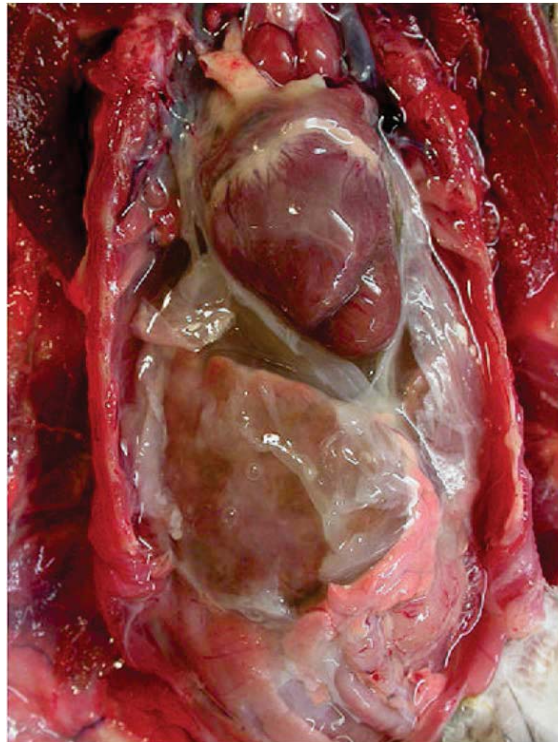


Figure 18: An avian liver that has severe cirrhosis in a 51 year-old grey parrot (Reavill & Dorrestein, 2010).

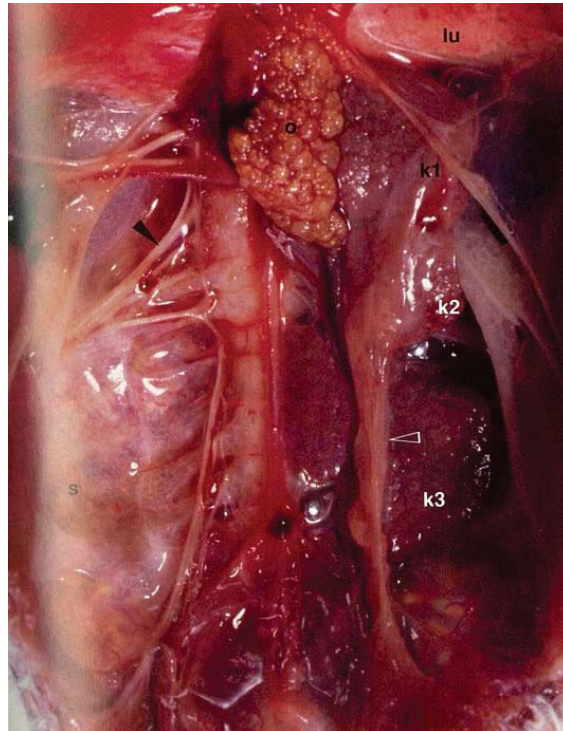


Figure 19: Normal avian kidneys which include (k1) cranial, (k2) middle, and (k3) caudal kidneys, also includes (lu) lung, and (o) ovary (Ritchie, Harrison, & Harrison, 1999).

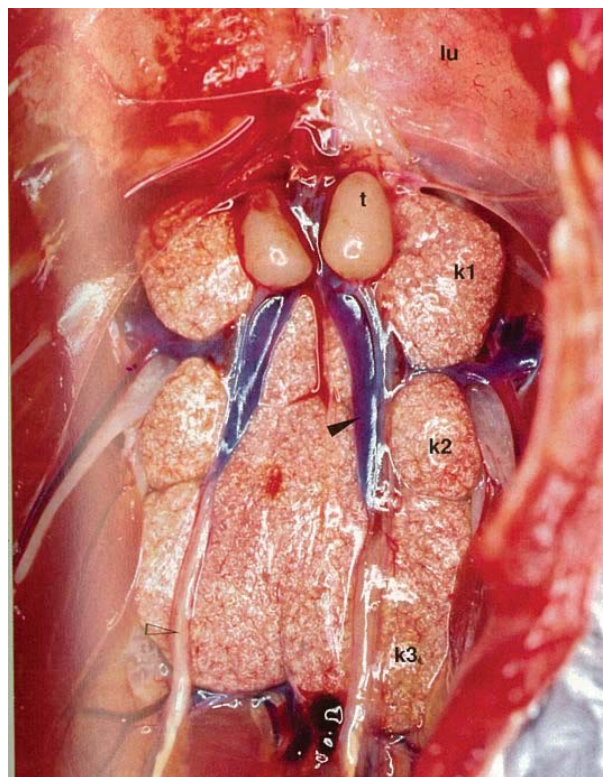


Figure 20: Avian kidneys that present with uric acid deposits (gout) which gives the speckled appearance. Anatomy of the image includes k1) cranial, (k2) middle, and (k3) caudal kidneys, also includes (lu) lung, and (t) testicles (Ritchie et al., 1999).

3.2. Age-related pharmacology of NSAIDs

The adverse effects of NSAIDs have been well documented over the years due to the COX-1 and COX-2 inhibition properties. The risk of developing gastrointestinal, liver or kidney adverse side effects can be attributed to several factors, including: age; medical history of the patient; the drug used combined with the dosage and duration of use (Bateman & Kennedy, 1995).

NSAIDs are used for a wide variety of conditions and diseases to reduce the pain experienced by the patient, regardless of their age (Litalien & Jacqz-Aigrain, 2001). With the increased prevalence of disease in the elderly, it is not uncommon for these patients to have comorbidities which increase the risk of adverse effects. Sadly, the adverse effects are more common and serious in this population. Therefore, it is necessary to be aware of the anatomical, physiological and biochemical changes that occur with age, and adjust these changes in order to safely administer various drugs (Klotz, 2009).

In humans, the incident rate of developing upper GI bleeding as a result of NSAIDs steeply increases with age, especially over the age of 60 (Bateman & Kennedy, 1995). The adverse GI effects can appear during the early stages of treatment, but in elderly patients long-term use also increases the chances of this occurring. These issues arise predominantly through the administration of oral or suppository NSAIDs which increase the risk of ulcers due to absorption occurring through the GI wall. Whereas topical administration is thought to

be the safest in terms of preventing adverse effects, the clinical efficacy of the drugs is reduced (Bateman & Kennedy, 1995).

In the hepatic system, the total liver volume and blood flow show a decrease by 25-35% and 40% respectively in older age (Turnheim, 2003). As a result, one of the major changes that is seen is the liver's ability to metabolise drugs. In the elderly there is a reduction of between 30-50% in the ability to clear the drugs during the phase I reactions. This is predominantly due to the changes in the hepatic blood flow, liver mass and hepatic endothelium, rather than the metabolising enzymes required to break down the drugs (Hilmer et al., 2007). But there is also evidence to suggest that due to liver disease there are changes in the presence and function of the enzymes required to alter the structure of the drugs in phase I. It has been found that the expression of various isoforms of the cytochrome P450 enzyme decline due to mild liver disease, in particular CYP2C19. Whereas, for patients who have chronic kidney disease regulation of these various CYP isoforms is directly related to the administered drug (Bosilkovska, Walder, Besson, Daali, & Desmeules, 2012; Velenosi & Urquhart, 2014). Whereas phase II reactions are maintained in healthy, fit older people but are reduced in those who are classified as frail (Hilmer et al., 2007).

With the renal system being another major site of drug processing, age-related changes in the renal system include: the overall kidney mass decreases due to the reduction of nephrons; and intra-renal vascular changes lead to reduced blood flow in the afferent arterioles entering the cortex and an overall decline of about 1% each year. The reduction

in plasma flow rate and GFR reduces by 20-50% between the ages of 20 and 90 (Turnheim, 2003), this results in the inability to reserve water and concentrate urine during periods of dehydration. But the plasma creatinine levels do not show any significant changes due to age, which is attributed to the overall loss of body mass during this stage of life (Turnheim, 2003). Furthermore, NSAID-induced acute renal failure can develop due to: haemodynamic changes; glomerular lesions; reduced GFR; reduced sodium and water excretion; hyperkalemia and hypertension (Musu et al., 2011). In healthy adults the incidence rate of developing NSAID-induced nephrotoxicity or acute renal failure is less than 1%, whereas, the elderly population is at a much higher risk due to normal age-related decline in kidney function but also has the added complication of other comorbidities like diabetes and congestive heart failure which put the renal system under more strain (Musu et al., 2011).

So as a precaution it is advised that the use of NSAIDs, regardless of age, should be used minimally if an individual has renal or hepatic insufficiencies, or is severely dehydrated, as this could lead to toxicity and could potentially cause irreversible liver or renal failure (Litalien & Jacqz-Aigrain, 2001).

The next section will be examining whether the changes mentioned in this section within the hepatic and renal systems which are attributed to the age of an individual show any evidence in altering the pharmacokinetics of NSAIDs.

3.3. Pharmacokinetics and age

As mentioned in the previous section the age-related structural and functional changes to the renal and hepatic system have the most effect on the pharmacokinetic parameters of drugs, including NSAIDs. Most of the 'age-related' changes in drug pharmacokinetics are often related to their current health status and whether they have pre-existing health issues which may contribute to the alteration in the pharmacokinetic parameters. Some of the age-related changes in the elderly are seen with the reduced ability for the GI tract to absorb the drug, as well as decreases in the drug binding to plasma protein, which ultimately affects the distribution within the body, but the efficacy of the drug remains intact (Hilmer et al., 2007).

The implications of these structural and functional changes due to age vary when examining the pharmacokinetic parameters. Numerous studies have produced conflicted results for the age-related changes in the absorption of drugs. There is an indication that there is no change in absorption of drugs that are able to permeate through the gastrointestinal epithelium, but the absorption of drugs in the intestinal epithelium which require specific carrier-mediated transport mechanisms occurs at a slower rate in the elderly (Turnheim, 2003). Overall, the changes in absorption seem to be related to the specific drug, not because of an overall decline due to age. It has been mentioned that many OA drugs undergo first-pass metabolism, and the rate at which this happens noticeably declines with age, which increases the bioavailability within the systemic system (Mangoni

& Jackson, 2004).

The major changes seen in response to aging is the distribution, more so the volume of distribution. The V_d is smaller in water soluble, polar drugs, as a result this leads to higher plasma drug concentrations. As a precaution, the loading dose of these drugs should be reduced to accommodate these changes. On the other hand, the V_d of lipid soluble non-polar drugs increases, which ultimately increases the half-life and clearance. Either change in the V_d can lead to the accumulation of the drugs within the body, causing the concentration levels to become toxic and increase the risks of the various adverse side effects (Mangoni & Jackson, 2004). An early study by Woolf and colleagues in 1983 looked at whether the pharmacokinetics of piroxicam differed due to age. Although they concluded that there was no conclusive evidence that the half-life, clearance rate or volume of distribution showed any difference between the different ages in this particular study. It was not until they compared their results with another study of piroxicam in young healthy adults that was conducted by Rodgers and colleagues, there was evidence to suggest that there was a significance difference between the two age groups in the pharmacokinetics of piroxicam (Woolf et al., 1983). The comparison of the pharmacokinetic parameters from the two studies is depicted in *Table 4* (Mangoni & Jackson, 2004; Woolf et al., 1983).

The production of plasma proteins like albumin and α 1-acid glycoprotein, which drugs bind to for transport, declines due to age. These proteins are essential in distributing drugs to the various tissues within the body (Bosilkovska et al., 2012). In the study by Woolf and colleagues, they found a significant difference in the albumin concentration between the elderly participants and the young participants, of 35.2 ± 2.8 g/L compared to 41.4 ± 2.6 g/L respectively (Woolf et al., 1983).

In terms of the pharmacokinetic significance, a reduction in plasma proteins increases the proportion of unbound drug to bound drug in the systemic system. As a result, the concentration of the drug that is transported into the cells increases along with the drug's increased ability to be distributed further amongst the various tissues in the body, all of which can alter the known V_d and clearance rate for that particular drug. A pharmacokinetic study conducted by Greenblatt and colleagues in 1986, found a similar result in the albumin concentration as Woolf and colleagues. Elderly males who were between 66 and 89 years old, had an average albumin concentration of 4.13 ± 0.07 gm/100mL, whereas, the younger males aged 25 to 48 years old had on average an albumin concentration of 4.53 ± 0.06 gm/100mL (Greenblatt et al., 1986). This study went on further to investigate the possible effects upon the pharmacokinetics of aspirin. The results showed that the percentage of unbound aspirin was significantly higher in the elderly participants than the younger participants, 10.8% compared with 8.5% respectively. This difference between the two age groups of unbound aspirin was related to the amount of albumin present in the plasma as the correlation between these two variables showed to be

statistically significant. These results did not appear to have any effect on the V_d of aspirin, however, there was a clear difference between the clearance rate of the two age groups, where the clearance of aspirin took significantly longer in the elderly participants than the younger participants (Greenblatt et al., 1986). Although this study was able to identify significant changes between elderly and young participants, the authors concluded that there are numerous possibilities for the changes or lack thereof in the pharmacokinetics of piroxicam that were not included in this study (Greenblatt et al., 1986). There are so many changes in the composition of the body as it ages which makes it hard to pinpoint the exact cause of changes in pharmacokinetics of any particular drug. For instance, in *Figure 21* it shows four changes in body composition as a human ages which can affect the pharmacokinetic information (Klotz, 2009).

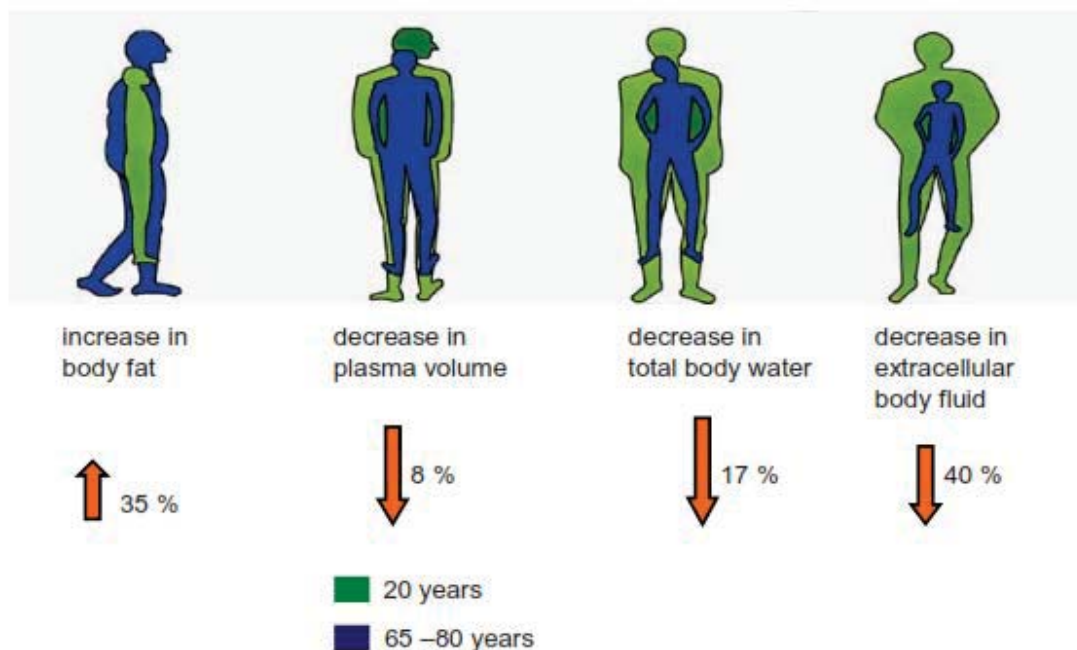


Figure 21: The change in body composition in young adults and elderly (Klotz, 2009).

Table 4: The mean \pm SD of pharmacokinetic parameters between elderly participants when administered piroxicam at 20 mg/kg (Woolf, Rogers, Bradbrook, & Corless, 1983) and young participants when administered piroxicam at 20mg/kg (Rodgers, 1981).

| Pharmacokinetic Parameter | Elderly participants (Woolf et al., 1983) | Young Participants (Rodgers, 1981) |
|---|--|---------------------------------------|
| Half-life (hours) | 73.4 \pm 24.6 | 52.9 \pm 20.1 |
| Clearance (mL.h ¹ .kg ¹) | 3.2 \pm 1.8 | 2.08 \pm 0.46 |
| Volume of distribution (L/kg) | 0.31 \pm 0.16 | 0.15 \pm 0.05 |

Conclusion

In conclusion, this section has outlined some of the issues which arise due to age and the possible effects upon the pharmacokinetics of NSAIDs. When looking at the life expectancies of various avian species, there are many species which can live up to 45 years old (Doneley, 2011; Reavill & Dorrestein, 2010). The extended life expectancy of avian species can be partly attributed to the advancement in veterinary interventions. Therefore, there will be a higher proportion of 'geriatric' birds requiring treatment, one of which is pain relief. In determining whether age is a factor in meloxicam pharmacokinetics will ultimately assist veterinarians in their care for avian species.

It is clear from this section and section 2.1 that in both the human population and avian species the hepatic and renal systems are crucial in processing and eliminating NSAIDs. Both humans and birds alike show changes in these two organ systems as they age. The changes seen are attributed to either natural age-related regression of the organs or the subject's increased susceptibility to conditions or diseases which can have primary or secondary effects upon these organs. As a result, the pharmacokinetics of a drug can be altered and can do further damage to these sensitive organs.

Unfortunately due to the lack of reliable studies it is hard to confidently examine or conclude if age has any true effect upon the pharmacokinetics of NSAIDs. There are only a few studies that have used human participants to investigate whether the age of the subject has an effect upon the pharmacokinetics of an NSAID, piroxicam which is in the same family of NSAIDs as meloxicam. However, these studies are extremely old and at the time these studies were conducted the concepts behind pharmacokinetics were still in their infancy. So it is hard to make any substantial conclusions from their studies on the possible affects age has upon the pharmacokinetics in human participants. As a result, it makes it hard make generalised assumptions that can be applied to avian species.

4. Chapter One conclusion

The use of NSAIDs have been used since the late 1800 's to alleviate pain. But it was not until the late 1900 's the mechanisms behind these drugs started to be uncovered. Although NSAIDs have been in circulation for decades, there is still a lot of information unanswered, especially concerning how they interact and distribute within the body. In pharmacology there is a subsection which investigates the spatial and temporal distributions of drugs, this is known as pharmacokinetics. The scope of pharmacokinetics is extensive and complex but extremely necessary when it comes to understanding and treating a wide variety of animals. The pharmacokinetics of NSAIDs is one area that still requires a lot of investigation, especially when considering the vast differences in physiology, biochemistry and metabolism in various animal groups. All of these differences fundamentally affect the pharmacokinetics of a particular drug. By investigating and documenting the pharmacokinetics of NSAIDs, it allows veterinarians to effectively treat patients with an appropriate drug and at the right dosage to target pain.

The current literature present concerning the pharmacokinetics of NSAIDs has been predominantly focused on various mammals even though the use of NSAIDs is popular in other animal groups such as avians. Very little investigation has been done into the pharmacokinetics of various NSAIDs in avian species, which presents the problem of being able to determine the correct dosages for the individuals. At this present time, the dosage of NSAIDs like meloxicam in birds is determined through extrapolating mammalian data to

predict the dosage based on weight. This method of treatment carries many risks for birds, including possible fatal overdoses due to their inability to metabolise and eliminate such a large quantity of drug. So it is evident that this gap in the literature needs to be filled through extensive studies in avian species in order to give veterinarians confidence in treating birds that is based on species-specific pharmacokinetic information but to also prevent such fatal outcomes for birds.

Simultaneously, other factors which may influence the pharmacokinetics of NSAIDs is also required to increase the overall information about these drugs. One of these factors is age. It is evident in humans, there are changes within their bodies which are attributed to the person's age. On a pharmacokinetic view point, there are 'age-related' changes in the hepatic and renal systems, both are crucial in body's ability to process drugs. These organ systems show deterioration in their general functions which can ultimately alter the pharmacokinetics of a drug which alters the pharmacodynamics (a drug's effect upon the body) of that particular drug. From examining 'age-related' changes within the body of both humans and birds, there are similar changes seen in key organs that process drugs. In addition, human studies have shown that the pharmacokinetics of various NSAIDs show significant differences due to age. It is for these reasons, that it is important to investigate whether the pharmacokinetics of NSAIDs in animals such as birds also show changes when age is a measured variable. By determining the possible effects that age contributes to the pharmacokinetics of meloxicam, it will further the information available to the veterinarians in order to treat animals like birds with a better understanding of their

underlying factors which contribute to the experience of pain and reducing it effectively.

5. Objectives

1. To describe the pharmacokinetics of meloxicam in ISA Brown chickens.
2. To investigate whether the age of the ISA Brown chickens have an effect on the pharmacokinetics of meloxicam.

This study aims to collect information on the pharmacokinetics of meloxicam in ISA Brown chicken to increase the information available in this area of scientific literature. In addition, this study will focus on determining if the age of a chicken could influence the pharmacokinetics of meloxicam. Better understanding of the mechanisms behind meloxicam in chickens will enable the data to be extrapolated to other avian species, and as a result, reduce the potential risks involved that come with extrapolating pharmacokinetic data from mammals for birds (Hunter et al., 2008).

The results from this study have gone beyond just adding to the pool of pharmacokinetic scientific literature. They go towards enhancing the welfare and quality of care for birds requiring veterinary treatment. The experience of pain is very subjective upon the individual, yet through years of research into the welfare of animals, the effect of pain upon an animal can be far-reaching and extensive depending on the origin of pain. By understanding the many factors that allow an animal to be relieved of pain, ultimately improves their overall welfare and promotes a positive life whether an animal is a pet, an endangered species or are production animals.

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Chapter Two

Pharmacokinetics of Meloxicam in
ISA Brown Chickens (*Gallus gallus
domesticus*).

6.1 Pharmacokinetics of Meloxicam in ISA Brown Chickens (Gallus gallus domesticus).

Abstract

Meloxicam is a popular Non-Steroidal Anti-Inflammatory drug (NSAID) worldwide for treating a wide variety of injuries. It is also used as the first line of treatment in injury prior to definitive diagnosis to reduce pain and distress in the animal. Although meloxicam is used to treat both mammals and avian species, there is very little information on the pharmacokinetics of meloxicam in avian species. As a result, when veterinarians administer meloxicam to birds, they are doing so basing the dosage off mammalian data. Due to this void in literature this study was designed to increase the basic pharmacokinetic knowledge in birds but to also determine if age affects the pharmacokinetics of meloxicam in ISA Brown chickens. Meloxicam was injected IV at 2 mg/kg in 20 healthy ISA Brown chickens (*Gallus gallus domesticus*). One group consisted of 10 ISA brown chickens that were 18 weeks old. The second group consisted of 10 ISA Brown chickens that were 24 months old. Serial blood samples were withdrawn from a catheterised vein from each ISA Brown chicken into a heparinised vial at 0, 10, 20, 30 minutes, 1, 4, 8, 10, 12 hours after the administration of meloxicam.

The pharmacokinetics for ISA Brown chickens were calculated using the non-compartmental model, which was analysed using the mean data from each group of ISA

Brown chickens. The elimination half-life, steady state volume of distribution and mean resident time were significantly higher in the 24 month old ISA Brown chickens compared to the 18 week old ISA Brown chickens. Overall, the results indicate that as an ISA Brown chicken ages the pharmacokinetics of meloxicam show some significant changes in crucial pharmacokinetic parameters. The differences in the pharmacokinetic parameters may ultimately affect the efficacy of meloxicam when treating 'geriatric' birds due to possible age-related health issues in the liver and kidneys, which are major organs involved in processing drugs.

6.2 Introduction

The experience of pain manifests in both physiological and behavioural changes which is considered to be a significant negative state for any animal to be in (Mellor & Stafford, 2002; Stafford & Mellor, 2002). By addressing the physiological aspect of pain and relieving it often leads to the animal exhibiting 'normal' behaviours which indicate a reduction of the negative state of the animal therefore, improving their overall welfare (Stafford & Mellor, 2002). Since the discovery and introduction into the commercial market, NSAIDs are a popular and effective treatment in controlling pain through the inhibition of prostaglandins after a tissue injury (Lierz & Korbel, 2012). A number of NSAIDs are commonly used in veterinary medicine as an alternative to the short lasting opioid drugs like butorphanol and morphine. Meloxicam has risen in popularity since it was first marketed as it remains an effective pain management drug whilst reducing the common side effects other NSAIDs can cause in mammals (Lierz & Korbel, 2012).

The pharmacokinetics of meloxicam has been studied in a variety of mammals including cattle (*Bos taurus*) (Coetzee, KuKanich, Mosher, & Allen, 2009), donkeys (*Equus africanus asinus*), horses (*Equus caballus*) (Sinclair et al., 2006), sheep (*Ovis aries*) (Stock, Coetzee, KuKanich, & Smith, 2013), cats (*Felis catus*) (Giraudel, Diquelou, Laroute, Lees, & Toutain, 2005), dogs (*Canis lupus familiaris*) (Caulkett, Read, Fowler, & Waldner, 2003), rabbits (*Oryctolagus cuniculus*) (Carpenter, Pollock, Koch, & Hunter, 2009) and rats (*Rattus*) (Aguilar-Mariscal, Patino-Camacho, Rodriguez-Silverio, Torres-Lopez, & Flores-Murrieta,

2007). As a result, the risk of adverse reactions is reduced significantly in many mammals because these studies have been able to determine the appropriate doses and dosing intervals which alleviate pain without causing overdoses. However, this cannot be said for the avian species requiring pain relief. The pharmacokinetic information on any NSAID including meloxicam is relatively unknown in avian species. To calculate the loading dose and dosing interval for a bird, it often requires the extrapolation from mammalian pharmacokinetic data through allometric scaling (*Refer to section 1.5*). One of the drawbacks in using this method, is it does not take into consideration the differences in physiology, metabolism and biochemistry of the avian species (Hunter & Isaza, 2002). Therefore, getting the right loading dose and dosing intervals is extremely difficult and must be done with caution as an error can prove fatal. One of the reasons meloxicam is used in avian species is because it has wide safety margins which reduces the extrapolating error (Lierz & Korbel, 2012).

Meloxicam pharmacokinetic studies that have been conducted in birds show a huge species-specific variation (Baert & de Backer, 2002a, 2002b; Lacasse et al., 2013; Molter et al., 2013; Naidoo et al., 2008). A study conducted by Baert and colleagues showed that meloxicam in chickens and pigeons which weighed between 0.45 and 2.2 kilograms, had a smaller volume of distribution compared to heavier birds. As a result, the clearance and elimination rate of meloxicam were faster in these lighter birds, regardless of a higher half-life (Baert & de Backer, 2002a). This assumption does not continue when other birds from other species are examined. For instance, birds in the same weight range like the red-tailed

hawks and great horned owls did not have the same relationship with the pharmacokinetic parameters. Instead, these birds had similar pharmacokinetic parameters to the ostriches and turkeys who had an average weight of 19 and 8 kg respectively (Baert & de Backer, 2002a; Lacasse et al., 2013). Due to the lack of literature of pharmacokinetics of meloxicam in birds, no significant relationship has been found to determine why there is such a huge variation between avian species. It is obvious that there are many factors involved in understanding the pharmacokinetics.

One factor that has not been considered in the scientific literature is whether age affects the pharmacokinetics of a drug, including NSAIDs like meloxicam in birds. With age, there is a general decline in the functions of many organs within the body. One of the major changes due to age is the ability of the liver to metabolise drugs. In elderly humans, changes in the hepatic blood flow, liver mass and hepatic endothelium seem to have the biggest effect on drug metabolism and elimination. Whereas, pre-existing renal disease affects the glomerular filtration rate and seems to be the only cause of age-related changes within the renal system. As a result, the elimination of drugs from the body is affected (Hilmer et al., 2007). The clearance of salicylate shows age-related changes, with clearance of the unbound salicylate taking a significantly longer time to clear in elderly humans (Greenblatt et al., 1986). A drug within the same family as meloxicam, known as piroxicam, also shows age-related changes. The volume of distribution, half-life and clearance in elderly human patients were markedly higher than the younger human patients (Woolf et al., 1983).

Human studies have shown that the age of participants influences the pharmacokinetics of common NSAIDs. From examining the 'age-related' changes within the body of both humans and birds, there are similar changes seen in key organs that process drugs. So there is a possibility that the changes in the pharmacokinetics of NSAIDs in humans may also be present in the ISA Brown chickens when using meloxicam (Doneley, 2011; Woolf et al., 1983). When looking at the life expectancies of various avian species, there are many species which can live up to 45 years old (Doneley, 2011; Reavill & Dorrestein, 2010). The extended life expectancy of avian species can be partly attributed to the advancement in their veterinary care, for instance, through better husbandry techniques, early surgical intervention and appropriate and targeted antibiotic treatment. Therefore, there will be a higher proportion of 'geriatric' birds requiring treatment, one of which is pain relief. For this study, meloxicam was injected IV at 2 mg/kg in 20 healthy ISA Brown chickens (*Gallus gallus domesticus*). One group consisted of 10 ISA Brown chickens that were 18 weeks old. The second group consisted of 10 ISA Brown chickens that were 24 months old. This study aims to collect information on the pharmacokinetic parameters of meloxicam in ISA Brown chicken to increase the information available in this area of scientific literature. In addition, this study will focus on determining whether the age of a chicken influences the pharmacokinetics of meloxicam. In determining whether age is a factor in meloxicam pharmacokinetics it will ultimately assist veterinarians in their care for avian species regardless of their age.

6.3. Experiment One: Pharmacokinetics of Meloxicam in 18 week old and 24 month old ISA Brown Chickens (Gallus gallus domesticus).

Study Design

This study was conducted on 20 healthy ISA Brown chickens (*Gallus gallus domesticus*). One group consisted of 10 ISA brown chickens that were 18 weeks old with an average body weight of 1.8 ± 0.2 kg. The second group consisted of 10 ISA Brown chickens that were 24 months old with an average body weight of 2.2 ± 0.2 kg.

All of the chickens were supplied from Hessels Poultry Farm (Levin, New Zealand). Once at the experimental facility, the Food Processing Unit Massey University (Palmerston North, New Zealand), the birds were individually housed in standard housing and maintenance conditions and allowed to acclimatise to the experimental conditions for one week prior to the commencement of this study. Each chicken had a 24 hour supply of fresh clean water and chicken feed which was produced on site at the Food Processing Unit Massey University (Palmerston North, New Zealand). All the chickens underwent a basic external health check by the chief applicant Dr Preet Singh, who deemed them all to be in a healthy condition and able to participate in this study. This study was approved by the Massey University Animal Ethics Committee (MUAEC). The MUAEC authorisation code assigned to this study is 14/27.

Drug Administration

The experiment was conducted over a four day period. On each day of the trial, five ISA Brown chickens were catheterised with a 22G catheter in a medial metatarsal vein. This was the site which the serial blood samples were taken from. Meloxicam (Metacam) 5 mg/mL (Boehringer Ingelheim, East Tamaki, Auckland, New Zealand) was injected intravenously into the medial metatarsal vein contralateral to the catheterised leg, at a dose of 2 mg/kg body weight using a 22 G needle.

Sample collection

Nine serial blood samples were collected from each ISA Brown chicken. The blood samples (1 mL each) were withdrawn from the catheterised vein into a heparinised vial at 0, 10, 20, 30 minutes, 1, 4, 8, 10, 12 hours after the injection of meloxicam. The blood sample taken at time zero was withdrawn from the catheterised metatarsal vein prior to the administration of meloxicam which was used as a control sample. To prevent the catheterised vein from clotting, after every blood withdrawal, 1 mL of a Sodium Chloride: Heparin solution was injected into the catheter. Before a blood sample was taken, a small amount of blood was removed from the catheter to ensure that there was no residue blood left in the catheter from the previous sample collection. The total amount of blood withdrawn from each bird was 10 mL, which is approximately 4% of the total blood volume of a 2 kg chicken. The samples were kept in ice immediately after collection and centrifuged

at 3000 rpm for 10 minutes. Plasma was pipetted out and kept at -20 °C until the day of analysis.

Reagents and solutions

Meloxicam injection was purchased from Boehringer Ingelheim New Zealand. The same solution of meloxicam was injected and used as the standard for the analysis as it does not contain any antioxidant or preservative. The working standard was prepared daily in mobile phase. Laboratory reagents used were methanol, hydrochloric acid and acetonitrile (Merck, New Zealand). Potassium dihydrogen phosphate, diethyl ether, and ortho-phosphoric acid were purchased from BDH Lab Suppliers (Poole, United Kingdom. HPLC grade ultrapure water was obtained using the Milli-Q PFplus system (Millipore Corporation, Darmstadt, Germany). The mobile phase contained 20 mM potassium dihydrogen phosphate (KH_2PO_4). Base stock solution was prepared by weighing 136g of 1M KH_2PO_4 in 1 litre of milli-Q water. KH_2PO_4 (20 mM) was prepared by mixing 20 mL of KH_2PO_4 in 980 mL of milli-Q water (Bae, Kim, Jang, & Lee, 2007).

Sample Analysis

The plasma samples were analysed by High Performance Liquid Chromatography (HPLC) using a LC-10AD HPLC pump, SIL-10AD auto-injector, DGU-14A de-gaser, SPD-M10A

Diode Array Detector (Shimadzu, Japan) and the chromatographs were analysed by the Shimadzu client server version 7.3.

The separation of meloxicam was achieved with a Phenomenex Luna 5u C18 (2) 100A 150 X 4.6 mm column at 40 °C. The mobile phase consisted of 20mM potassium dihydrogen phosphate: acetonitrile (60:40) pH 3.5 with a flow rate of 1 mL/min under isocratic conditions. The DAD detector was set at 355nm wavelength. The Lowest Limits of Quantification was 31.25 ng/mL. The specificity of the method was confirmed by the absence of any peaks at the same retention time in the blank plasma after following the same extraction method (Bae et al., 2007).

Sample Preparation

The plasma samples were prepared by a liquid-liquid extraction (LLE) procedure. A 300 µl aliquot of sample was diluted with 100 µl of 5M hydrochloric acid. After a brief vortex 6 ml of diethyl ether was added and each sample was vortex mixed for 30 seconds. All the samples were centrifuged at 2500 g for 10 minutes, the supernatant was removed and transferred into a clean glass test tube. The samples were evaporated to dryness under a gentle stream of air at 40 °C and reconstituted with 200 µl of mobile phase. The injection volume was 50 µl and each sample was auto-injected two times in the HPLC system (Bae et al., 2007).

Pharmacokinetic analysis

The non-compartmental model was analysed using Pk Solver excel Macros (Zhang et al., 2010). In order to calculate the various pharmacokinetic parameters, the concentration-time data from each chicken was combined in order to produce a mean value, for both the 18 week old ISA Brown chickens and the 24 month old ISA Brown chickens. This data was used to produce the pharmacokinetic parameters: area under the curve ($AUC_{(0-t)}$), area under the curve from time zero to infinity ($AUC_{(0-\infty)}$), area under the first moment curve ($AUMC_{(0-t)}$), (C_l), mean residence time (MRT), half-life ($t_{1/2}$), and volume distribution at a steady state (V_{ss}).

Results

Method validation was conducted through analysing the recovery of meloxicam after the liquid-liquid extraction method, detailed in the section *sample preparation* on page 95. The standard curve for meloxicam was made by spiking blank plasma with meloxicam standard. The recovery of meloxicam in mobile phase was detected at the levels of 1000, 250, 62.5, 31.25, 15.625 and 7.81 ng/mL (*Figure 22*). The results from the validation studies of meloxicam produced a standard curve with a R^2 value of 95.36%. The unknown concentrations were calculated from the standard curve by linear regression using Prism 5 for Windows (Graphpad Software Inc, La Jolla, CA, USA). To maintain accuracy a standard curve was constructed before and after every run of samples using meloxicam standard in mobile phase.

The chromatographs for meloxicam in the 18 week old ISA Brown chickens during the first 30 minutes, 1 to 8 hours, and 10 to 12 hours, including blank plasma after the IV administration are shown in *Figures 23, 24 and 25* respectively. The absence of any peaks in the blank plasma at the same retention time in the other samples shows the specificity of this method to identify meloxicam. The semi-log plot for all the time points are shown in *Figure 26*. The pharmacokinetic parameters were analysed using the non-compartmental model, as the Akaike Information Criterion (AIC) values from the data fitted the curve better when compared to the Bayesian Information Criterion (BIC) values. The results from

the non-compartmental model of meloxicam in the 18 week old ISA Brown chickens are summarised in *Table 5*.

The pharmacokinetic parameters show that in the 18 week old ISA Brown the half-life ($t_{1/2}$) of meloxicam was 3.07 ± 0.20 hours. The overall mean residence time (MRT) of meloxicam was 4.45 ± 0.47 hours. The volume of distribution at a steady state (V_{ss}) was 0.0255 ± 0.009 mL/kg and clearance was 0.0056 ± 0.0014 mL/hr.

The chromatographs for meloxicam in the 24 month old ISA Brown chickens during the first 30 minutes, 1 to 8 hours, and 10 to 12 hours, including blank plasma after the IV administration are shown in *Figures 27, 28 and 29* respectively. The absence of any peaks in the blank plasma at the same retention time in the other samples shows the specificity of this method to identify meloxicam. The semi-log plot for the time points are shown in *Figure 30*. The pharmacokinetic parameters were analysed using the non-compartmental model. The non-compartmental model of meloxicam in 24 moth old ISA Brown chickens are summarised in *Table 6*.

The pharmacokinetic parameters show that in the 24 month old ISA Brown chickens the half-life ($t_{1/2}$) of meloxicam was 7.652 ± 3.03 hours. The overall mean residence time

(MRT) of meloxicam was 9.140 ± 2.99 hours. The volume of distribution at a steady state (V_{ss}) was 0.062 ± 0.030 mL/kg and clearance was 0.00639 ± 0.0015 mL/hr.

The combined concentration time curve between the 18 week old ISA Brown chickens and the 24 month old ISA Brown chickens is shown in *Figure 31*. An unpaired T-Test was performed to analyse whether there was any age-related differences in the pharmacokinetic parameters of 18 week old ISA Brown chickens and the 24 month old ISA Brown chickens, which is shown in *Table 7*. The 24 month old ISA Brown chickens' elimination half-life ($t_{1/2}$) was significantly higher ($p=0.0141$) when compared to the 18 week old ISA Brown chickens. The volume distribution at a steady state and mean residence time were also significantly higher $p=0.0373$ and $p=0.0127$ respectively in the 24 month old ISA Brown chickens when compared to the 18 week old ISA Brown chickens.

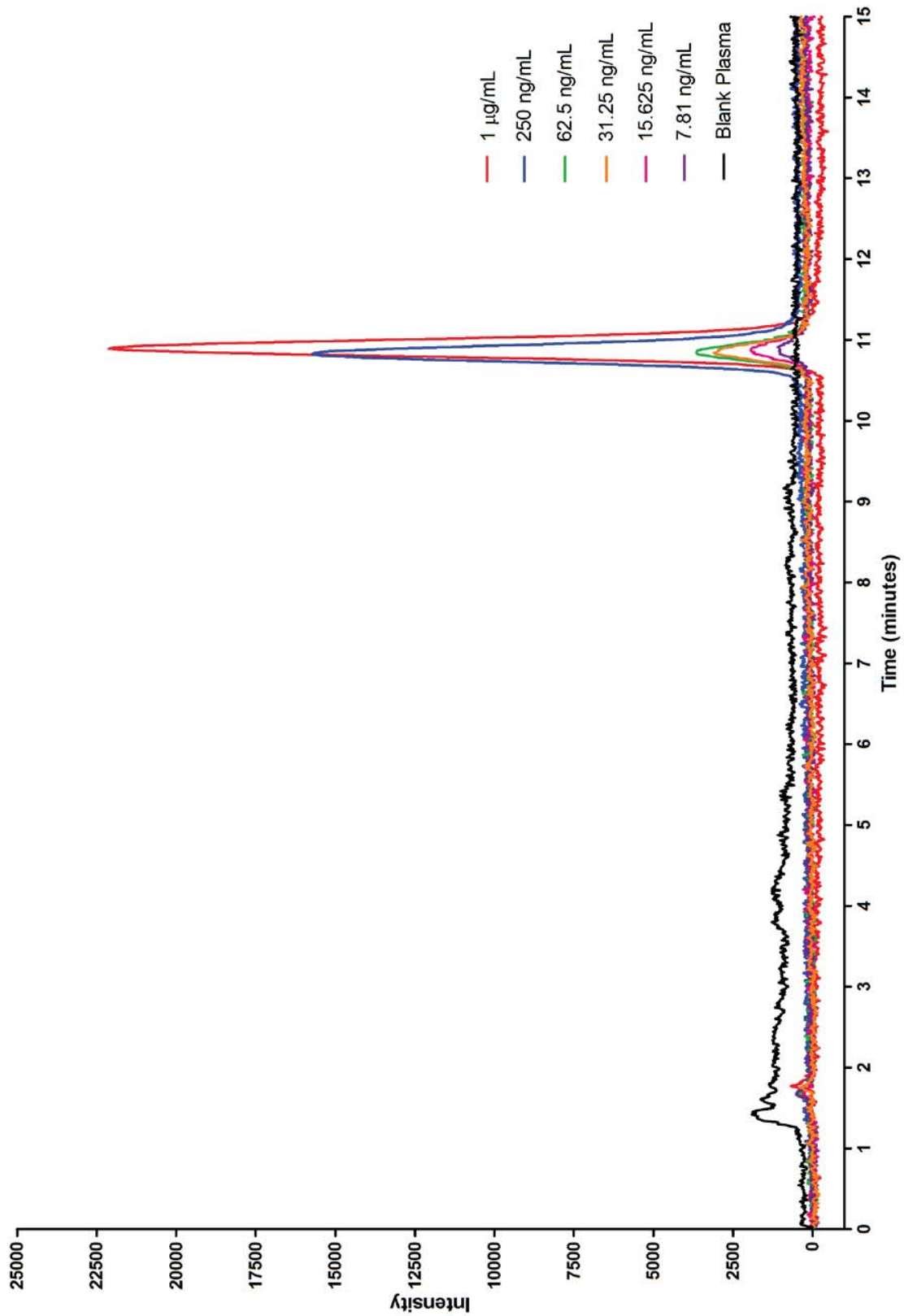


Figure 22: Chromatograph showing meloxicam standard solutions from 1 µg/ml to 7.81 ng/ml and blank plasma in mobile phase.

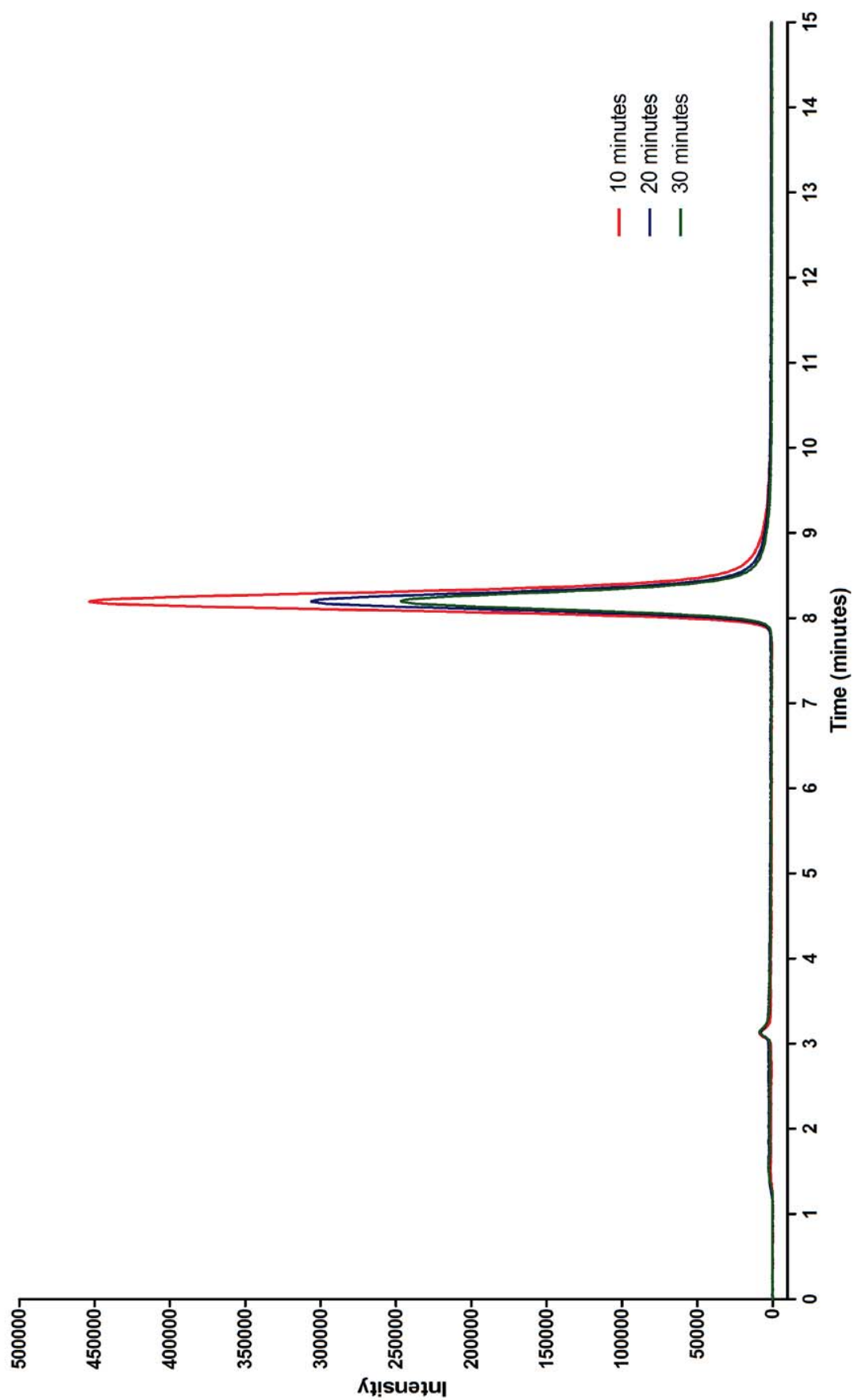


Figure 23: Chromatograph showing meloxicam peak after the first 30 minutes of intravenous injection at 2 mg/kg dose rate in 18 week old ISA Brown chickens.

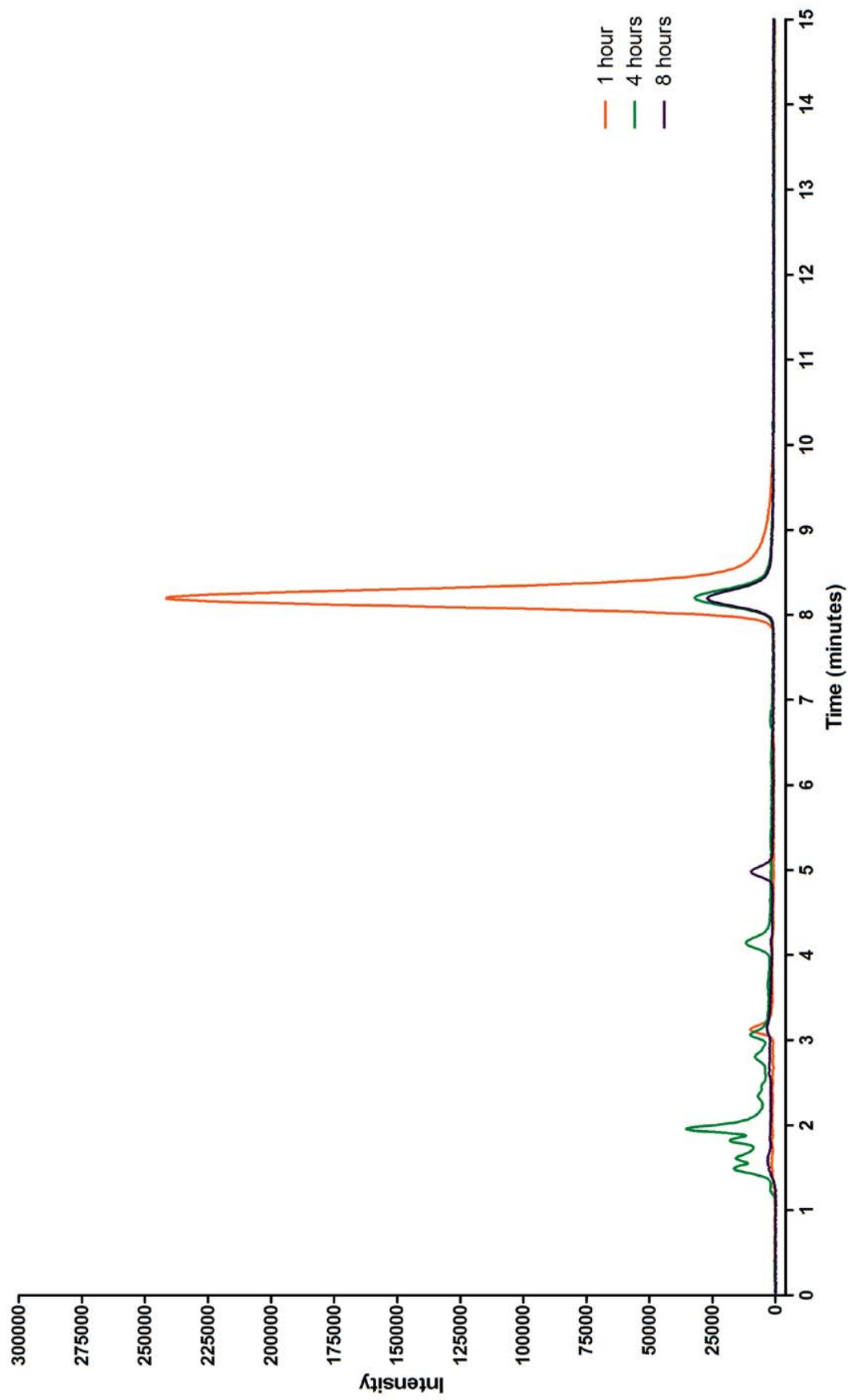


Figure 24: Chromatograph showing meloxicam peak between the first hour and eight hours after the intravenous injection at 2 mg/kg dose rate in 18 week old ISA Brown chickens.

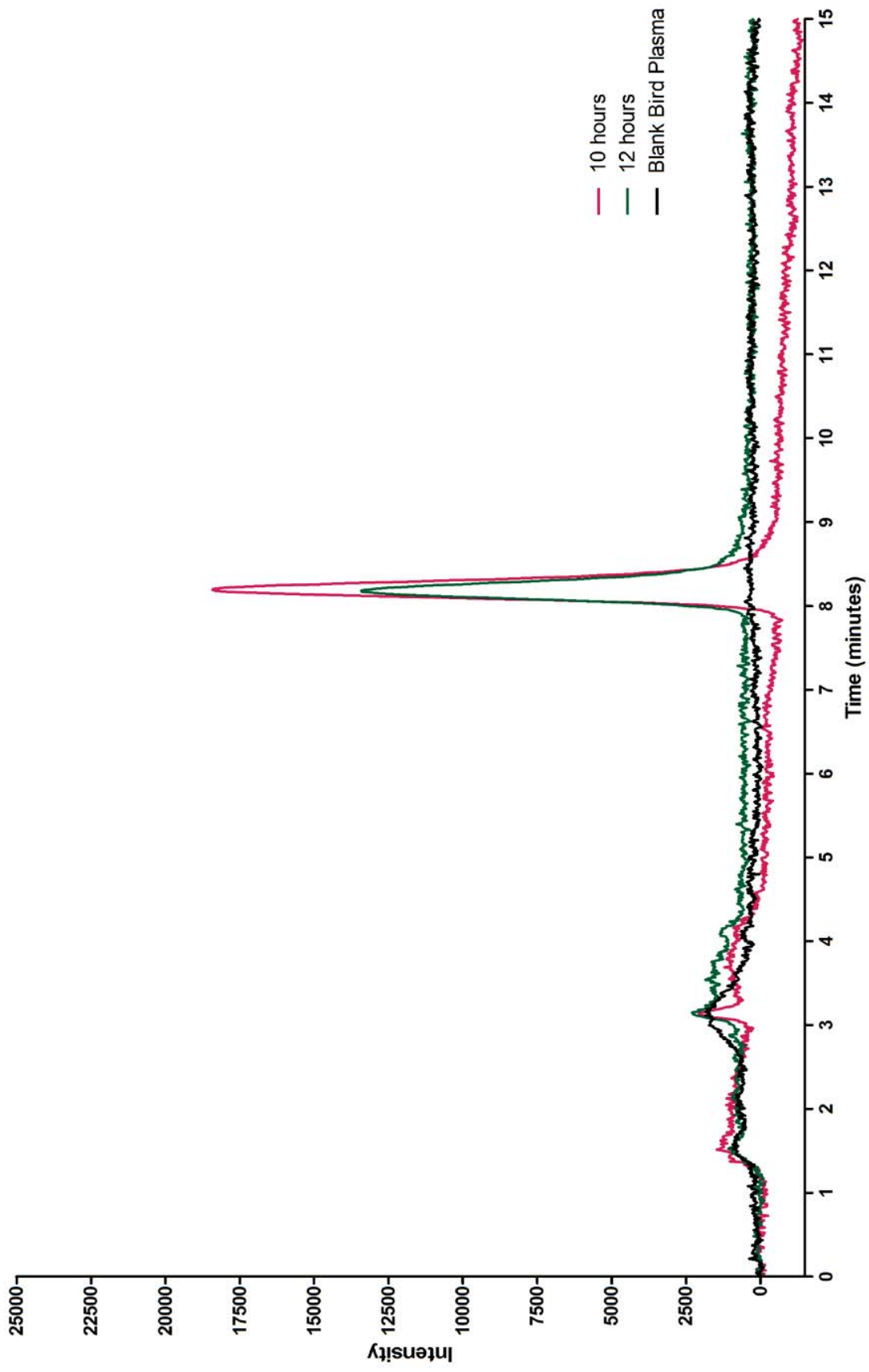


Figure 25: Chromatograph showing meloxicam peak 10 and 12 hours after the intravenous injection at 2 mg/kg dose rate in 18 week old ISA Brown chickens. The blank bird plasma does not show a retention peak at the same retention time as meloxicam following the same extraction procedure.

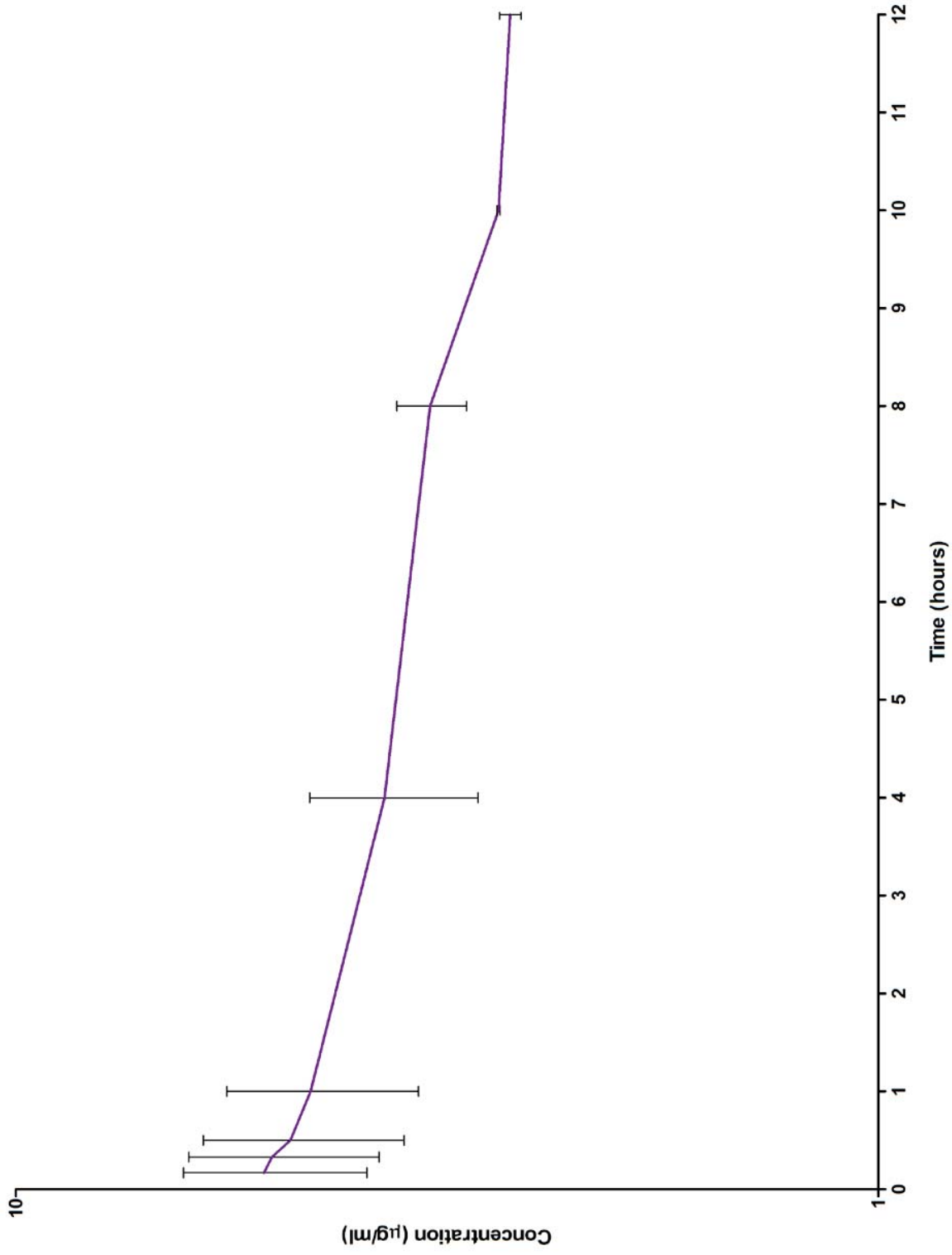


Figure 26: Semi-log plot of concentration time curve for meloxicam after intravenous administration at 2 mg/kg in 18 week old ISA Brown chickens. Each data point represents mean of 10 chicken's \pm SEM, and the total of 10 chickens were used in this study.

Table 5: Non-compartmental analysis of meloxicam in 18 week old ISA Brown chickens. Meloxicam was injected at 2 mg/kg intravenously. All of the parameters were calculated from the mean of 10 concentration time curves from the mean pool samples \pm SD.

| Parameters | Units | 18 week old ISA Brown chickens |
|---|----------------------|---------------------------------------|
| AUC _(0-t) | ng/mL.h | 608.8 \pm 134.02 |
| AUC _(0-∞) | ng/mL.h | 666.61 \pm 133.72 |
| AUMC _(0-∞) | ng/mL.h ² | 2925 \pm 416.4 |
| C _L | mL/hr | 0.0056 \pm 0.0015 |
| MRT | hours | 4.45 \pm 0.47 |
| t _{1/2} | hours | 3.07 \pm 0.20 |
| V _{ss} | (mg)/(ng/mL) | 0.0255 \pm 0.009 |

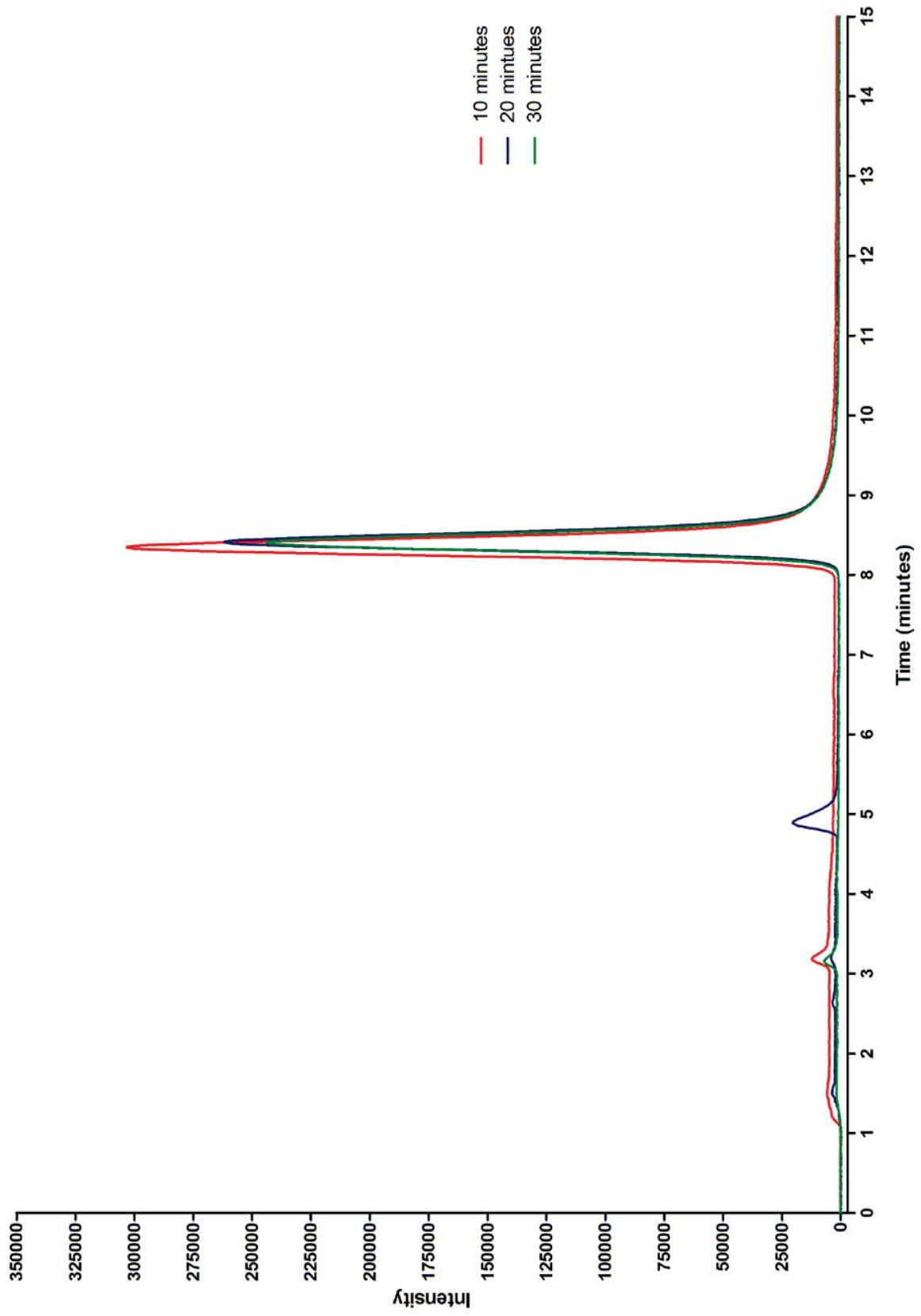


Figure 27: Chromatograph showing meloxicam peak after the first 30 minutes of intravenous injection at 2 mg/kg dose rate in 24 month old ISA Brown chickens.

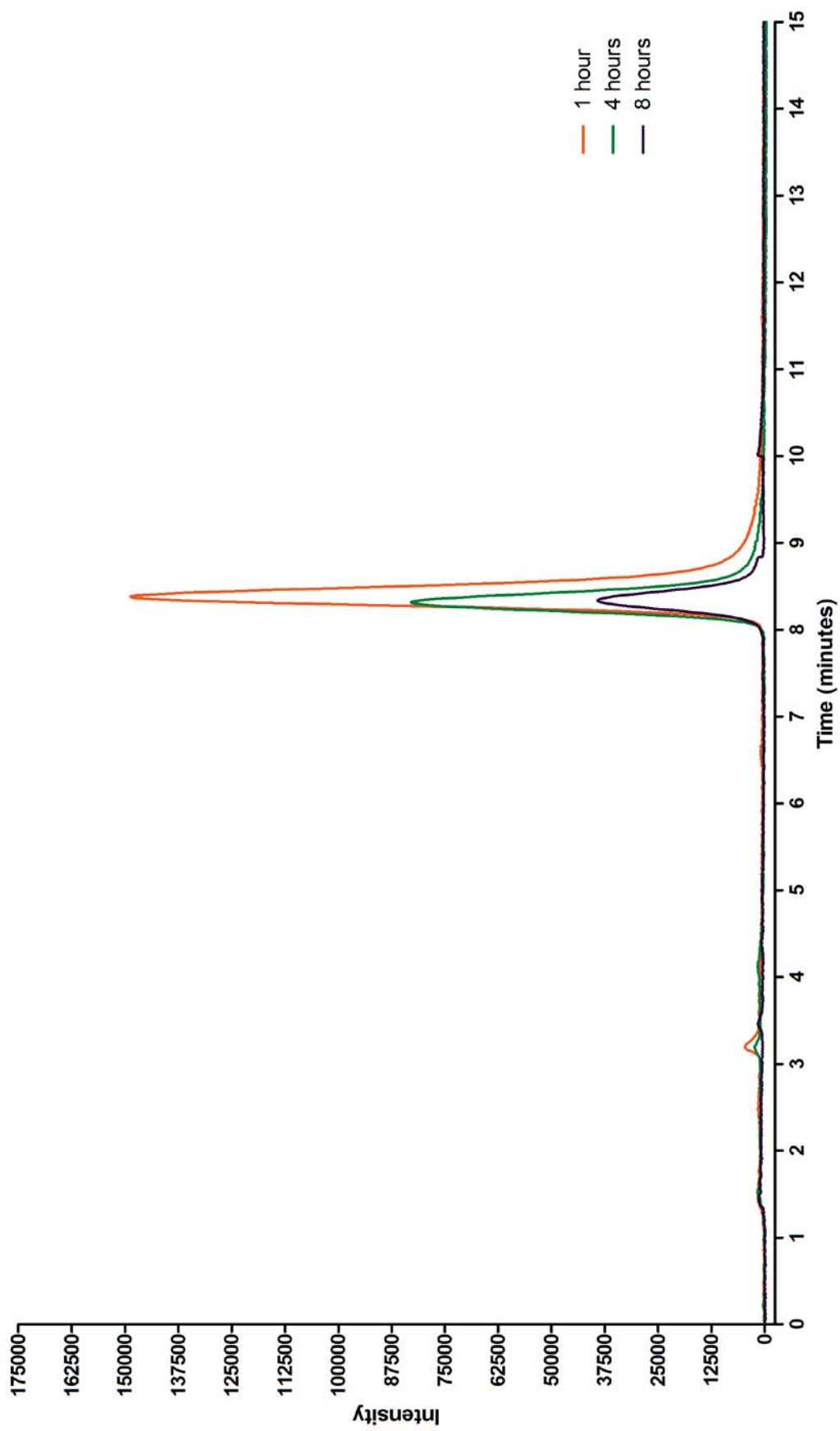


Figure 28: Chromatograph showing meloxicam peak between the first hour and eight hours after the intravenous injection at 2 mg/kg dose rate in 24 month old ISA Brown chickens.

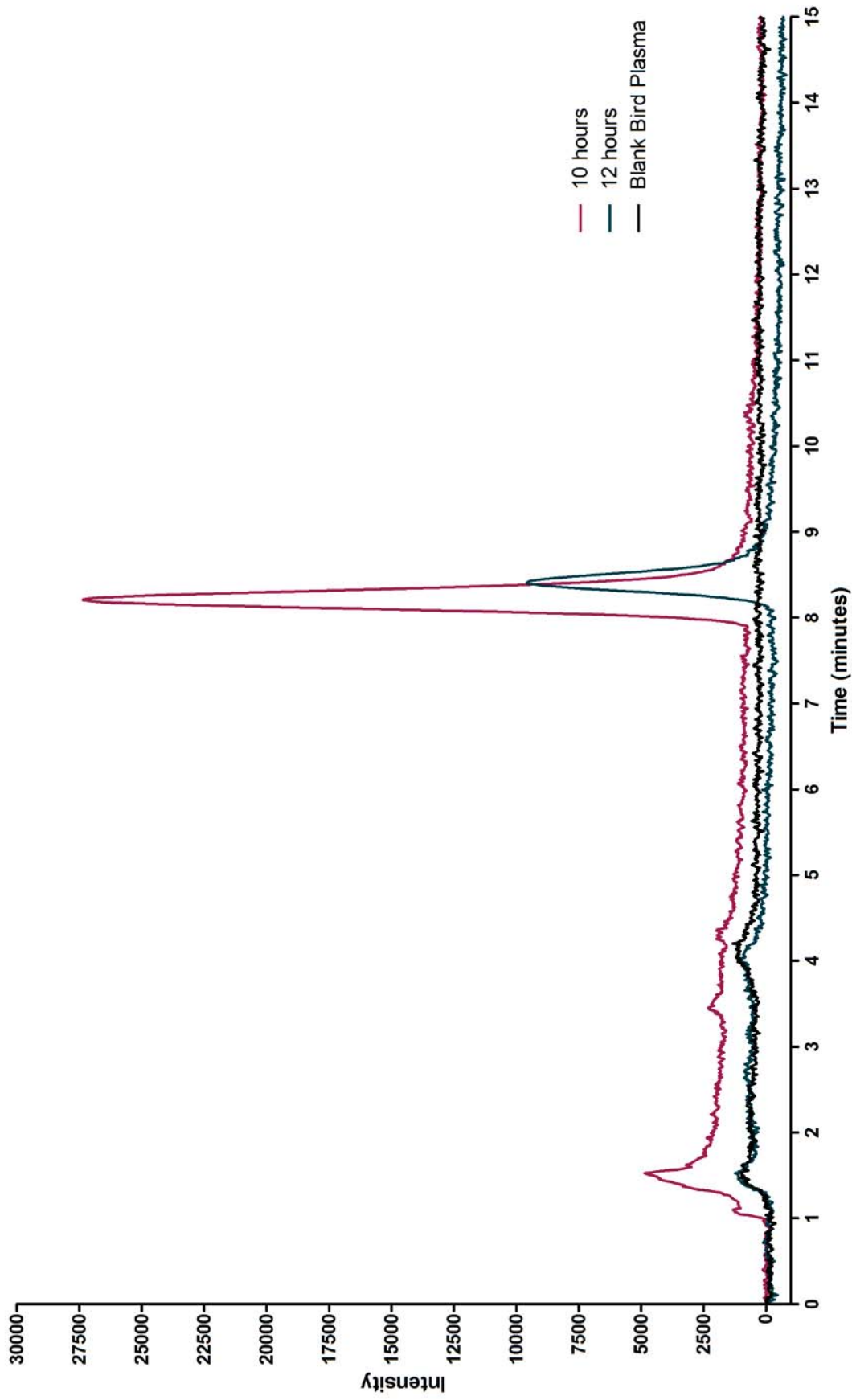


Figure 29: Chromatograph showing meloxicam peak 10 and 12 hours after the intravenous injection at 2 mg/kg dose rate in 24 month old ISA Brown chickens. The blank bird plasma does not show a retention peak at the same retention time as meloxicam following the same extraction procedure.

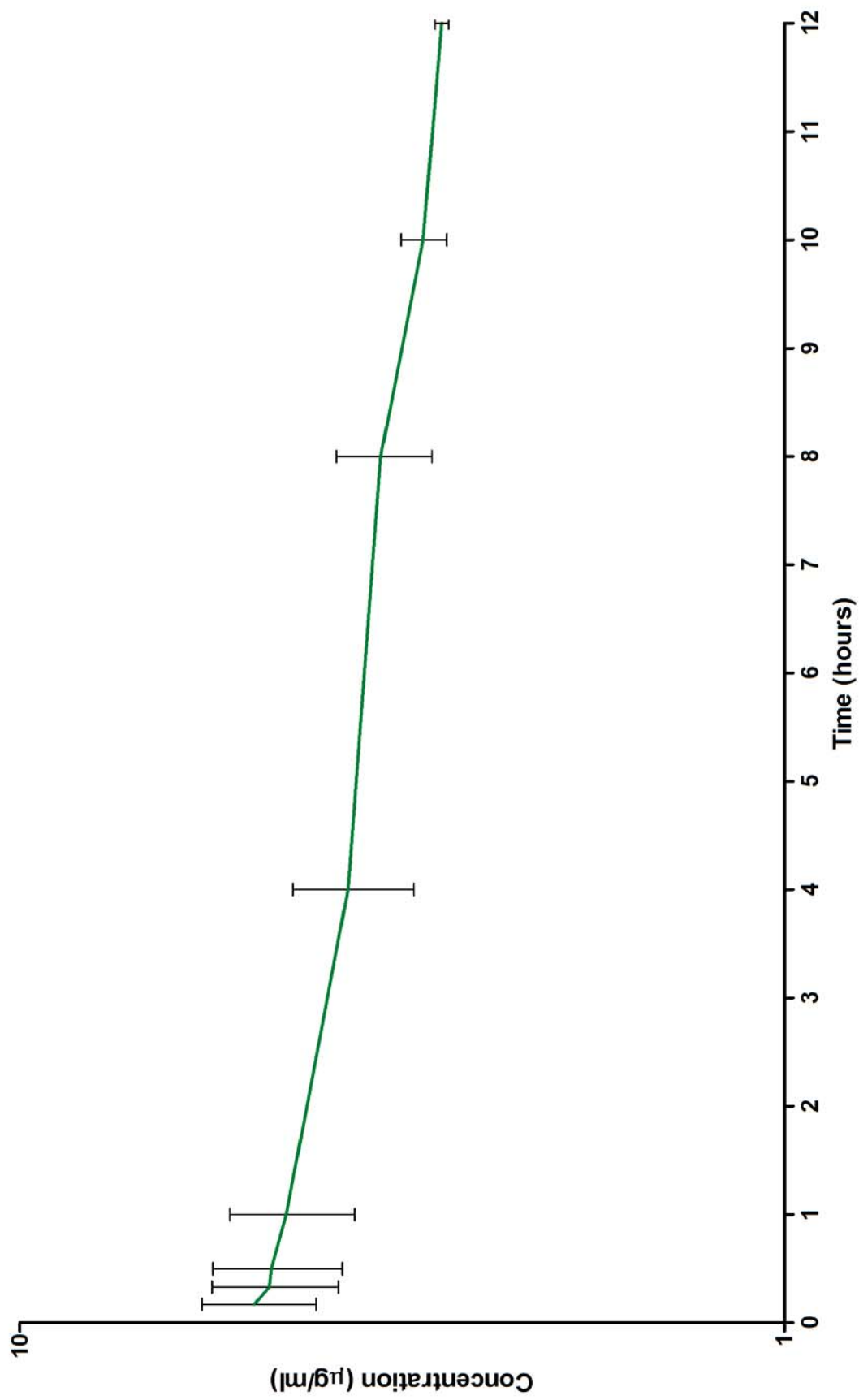


Figure 30: Semi-log plot of concentration time curve for meloxicam after intravenous administration at 2 mg/kg in 24 month old ISA Brown chickens. Each data point represents mean of 10 chickens \pm SEM, and the total of 10 chickens were used in this study.

Table 6: Non-compartmental analysis of meloxicam in 24 month old ISA Brown chickens. Meloxicam was injected at 2 mg/kg intravenously. All of the parameters were calculated from the mean of 10 concentration time curves from the mean pool samples \pm SD.

| Parameters | Units | 24 month old ISA Brown chickens |
|---|----------------------|---------------------------------|
| <i>Non-Compartmental</i> | | |
| AUC _(0-t) | ng/mL.h | 514.60 \pm 93.15 |
| AUC _(0-∞) | ng/mL.h | 701.64 \pm 129.89 |
| AUMC _(0-∞) | ng/mL.h ² | 6498 \pm 2716 |
| C _L | mL/hr | 0.0062 \pm 0.0015 |
| MRT | hours | 9.140 \pm 2.99 |
| t _{1/2} | hours | 7.652 \pm 3.03 |
| V _{ss} | (mg)/(ng/mL) | 0.062 \pm 0.030 |

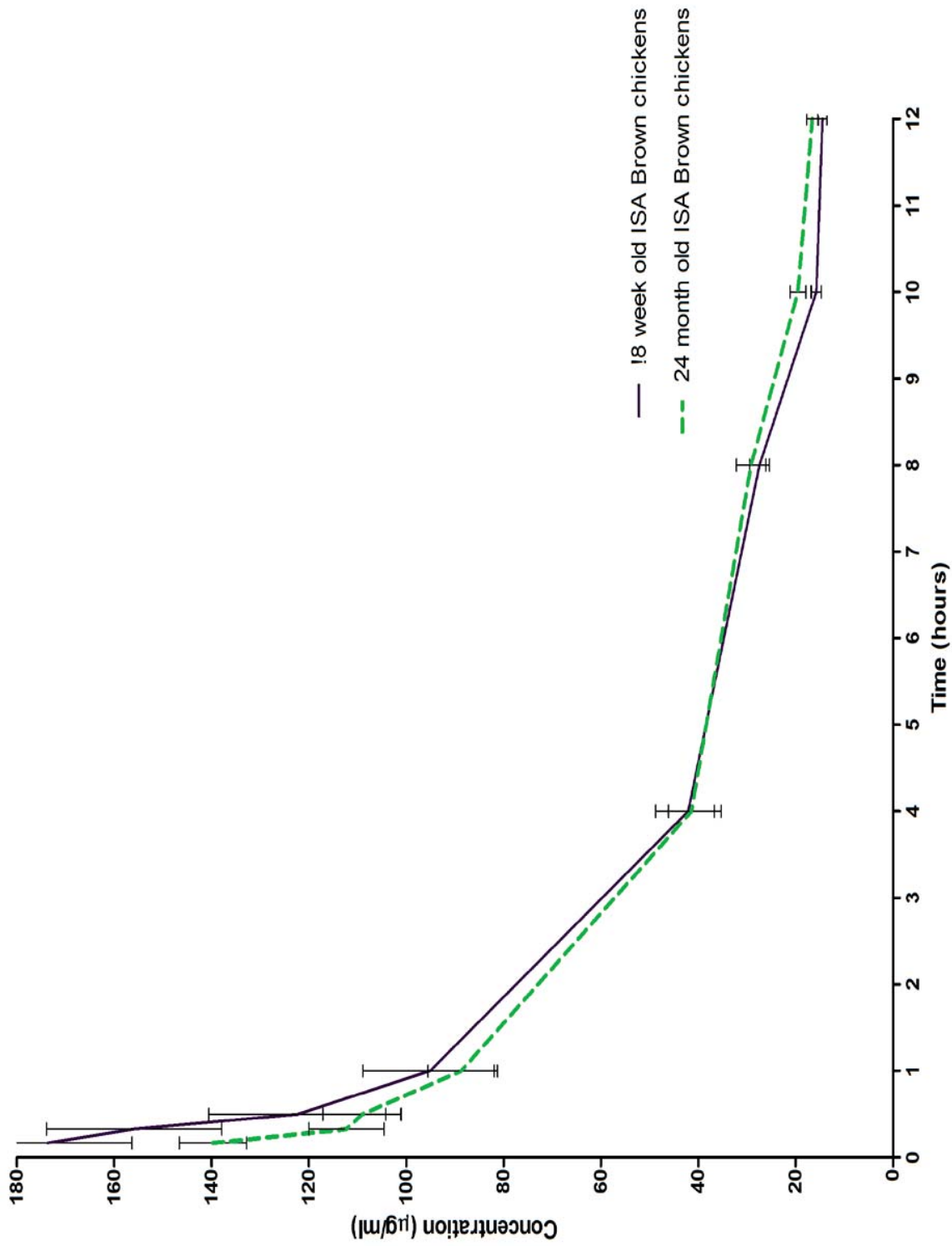


Figure 31: Concentration time curve for meloxicam after intravenous administration at 2 mg/kg in 18 week old ISA Brown chickens and 24 month old ISA Brown chickens. Each data point represents mean of 10 chicken's \pm SEM, and the total of 20 chickens were used in this study.

Table 7: Non-compartmental analysis of meloxicam in 18 week old ISA Brown chickens and 24 month old ISA Brown chickens. Meloxicam was injected at 2 mg/kg intravenously. All of the parameters were calculated from the mean of 10 concentration time curves from the mean pool samples \pm SD.

| Parameters | Units | 18 week old ISA Brown chickens | 24 month old ISA Brown chickens | P values |
|----------------------|--------------|--------------------------------|---------------------------------|----------|
| AUC _(0-t) | ng/mL.h | 608.8 \pm 134.02 | 514.60 \pm 93.15 | 0.195 |
| C _L | mL/hr | 0.0056 \pm 0.0015 | 0.0062 \pm 0.0015 | 0.403 |
| MRT | hours | 4.45 \pm 0.47 | 9.140 \pm 2.99 | 0.0127* |
| t _{1/2} | hours | 3.07 \pm 0.20 | 7.652 \pm 3.03 | 0.0141* |
| V _{ss} | (mg)/(ng/mL) | 0.0255 \pm 0.009 | 0.062 \pm 0.030 | 0.0373* |

Significant P values denoted with *

Discussion

In pharmacokinetics the distribution of a drug within the body can be analysed through four key principles: absorption, distribution, metabolism and elimination. It is from these principles that the core pharmacokinetic parameters were developed in order to analyse particular drugs (Foster, 2007; Jang et al., 2001). From pharmacological and pharmacokinetic studies, the properties of meloxicam make it a popular NSAID to use amongst mammals as an analgesic. Due to the efficacy as pain relief, meloxicam is also used in avian species. Unfortunately, due to the lack of pharmacokinetic literature on meloxicam specifically in avian species, the administration of meloxicam is based off extrapolated mammalian data (Hawkins, 2006). In addition, the limited avian pharmacokinetic data of meloxicam makes it harder to identify whether the properties of meloxicam are similar across various avian species and if not, how they differ in order for veterinarians to adjust the administration and use of meloxicam for specific species. This lack of knowledge in this subject continues to make it difficult for the veterinarians when it comes to administering meloxicam in birds and being able to effectively manage the pain and at the same time reduce the risk of major side effects for the individual.

In humans, the age of a person must be taken into consideration when prescribing and administering any drug. In particular, careful attention is required when dealing with elderly patients due to the functional decline seen in their hepatic and renal organ systems,

which are both fundamental in determining the pharmacokinetics of a particular drug and their associated pharmacodynamic properties (Bosilkovska et al., 2012; Musu et al., 2011; Turnheim, 2003). Although there has not been much exploration into the area of aging in birds and the associated effects that occur in older age, they show a similar decline as humans in these key organs and are more susceptible to diseases and conditions which can affect their overall health (Doneley, 2011; Holmes, 2004). In spite of this, current literature shows no direct investigation into what degree, if any, age plays in the pharmacokinetics of any drug, let alone analgesics such as meloxicam.

In this study, meloxicam was injected intravenously at 2 mg/kg in 20 healthy ISA Brown chickens (*Gallus gallus domesticus*), where one group consisted of 10 ISA Brown chickens that were 18 weeks old and second group which consisted of 10 ISA Brown chickens that were 24 months old. The objectives for this study were to firstly to describe the pharmacokinetics of meloxicam in ISA Brown chickens and secondly to investigate whether the age of the ISA Brown chickens has an effect on the pharmacokinetics of meloxicam.

The results from this study shows that the pharmacokinetics of meloxicam in chickens varies with age as observed in other published data on humans. In this study, the 18 week old ISA Brown chickens had a half-life ($t_{1/2}$) was similar to other chicken data from two different studies conducted by (Baert & de Backer, 2002a, 2002b), see table 8.

Whereas, the 24 month old ISA Brown chickens had a half-life which was remarkably higher compared to the other chicken data but similar to the Hispaniolan Amazon parrots (*Amazona ventralis*) (Molter et al., 2013). Comparison of the results from this study with the data from other avian species, both the 18 week old and 24 month old ISA Brown chickens had similar clearance rate to chickens in other studies (Baert & de Backer, 2002a, 2002b). In addition, the results from this study maintain the lower clearance rate when compared with other avian species such as the Red-tailed Hawks (*Buteo jamaicensis*) (Lacasse et al., 2013) and Ostriches (*Struthio camelus*) (Baert & de Backer, 2002a), see table 8.

Furthermore, the mean residence time (MRT) of meloxicam in the 18 week old ISA Brown chickens was similar to other chicken data (Baert & de Backer, 2002a, 2002b) and pigeons (*Columba livia*) (Baert & de Backer, 2002a). However, the MRT results from the 24 month old ISA Brown chickens were very different when compared with other chicken data (Baert & de Backer, 2002a, 2002b) and all the other avian species referenced in Table 8. The difference in 24 month old ISA Brown MRT results could be due to the individual variability that occurs due to the difference in age and body weight of each bird. In addition, the unique anatomical, physiological, biochemical and metabolism of each individual also contributes to the overall population variability in each study and the differences in results. Furthermore, it is well documented that with the advancement in age, there is impairment and decline in various organ functions, especially concerning the

liver and kidneys (Holmes & Ottinger, 2005; Klotz, 2009; Mangoni & Jackson, 2004). A decline in the function of these organs contributes to a decline in the overall metabolism of an individual. This decline will ultimately affect the pharmacokinetics of any drug. Therefore, the higher MRT and elimination half-life found in the 24 month old ISA Brown chickens could be partially attributed to a change in their metabolism.

The volume distribution at a steady state (V_{ss}) also shows consistency with the results from other avian studies. In particular, the V_{ss} of the 24 month old ISA Brown chickens was similar to the data found in chickens (*Gallus gallus domesticus*), ducks (*Anas platyrhynchos*) and turkeys (*Meleagris gallopavo*) (Baert & de Backer, 2002a). However, the V_{ss} in the 18 week old ISA Brown chickens was smaller compared to any other avian species that it was compared against, see table 8. There are several possibilities for this difference in results. Firstly, to analyse the pharmacokinetics of meloxicam this study used the non-compartmental model which calculates the V_{ss} , whereas, the other studies used either a one compartmental model or two compartmental model which calculates the apparent volume distribution (V_d) (the difference explained in section 2.4 on page 58). The values for V_d and V_{ss} are calculated from equations which draw the data from different variables, therefore, this could account for the difference seen in the 18 week old ISA Brown chickens compared with the other data. For instance, the apparent V_d is calculated by dividing the dose of the drug over the maximum concentration of the drug (C_{max}) (see equation in section 2.2.3 on page 46), whereas, V_{ss} is determined by multiplying the

clearance rate (C_i) with the mean residence time (MRT) (see equation in section 2.4 on page 58) (Riviere, 2011b). Secondly, the average weights of the birds in the other studies were similar to that of the 24 month old ISA Brown chickens. In this study, there was a clear difference in the weight between the 18 week old ISA Brown chickens and the 24 month old ISA Brown chickens, 1.8 ± 0.2 kg and 2.2 ± 0.2 kg respectively. This could indicate that the age of the birds in the other studies may have been a similar age as the 24 month old ISA Brown chickens or older, consequently, this could attest for why the results are more consistent with the other avian data. However, none of the studies recorded the average age of the birds involved in each study, so unable to adequately link these variables together.

Table 8: Comparison of the mean \pm SD of the pharmacokinetic parameters of meloxicam from this study and with various bird species in the literature.

| Author | Species | Weight (kg) | Dose (mg/kg) | V _{ss} (mg/ng/mL) | t _{1/2} (hours) | MRT (hours) |
|--------------------------|---------------------------------|-----------------|--------------|----------------------------|--------------------------|------------------|
| This study | 18 week old ISA Brown chickens | 1.8 \pm 0.2 | 2 | 0.0255 \pm 0.009 | 3.07 \pm 0.20 | 4.45 \pm 0.47 |
| This study | 24 month old ISA Brown chickens | 2.2 \pm 0.2 | 2 | 0.062 \pm 0.030 | 7.652 \pm 3.03 | 9.140 \pm 2.99 |
| Baert & de Backer, 2002a | Chicken | 2.2 \pm 0.2 | 0.5 | 0.058 \pm 0.005 | 3.21 | 4.41 \pm 0.84 |
| Baert & de Backer, 2002b | Broiler Chicken | 2.2 \pm 0.2 | 0.5 | 0.117 \pm 0.01 | 3.2 | 4.41 \pm 0.84 |
| Baert & de Backer, 2002a | Ostrich | 19 \pm 6 | 0.5 | 0.59 \pm 0.19 | 0.5 | 0.41 \pm 0.25 |
| Baert & de Backer, 2002a | Duck | 3 \pm 0.8 | 0.5 | 0.065 \pm 0.017 | 0.72 | 0.77 \pm 0.2 |
| Baert & de Backer, 2002a | Turkey | 8 \pm 1.7 | 0.5 | 0.079 \pm 0.015 | 0.99 | 1.47 \pm 0.27 |
| Baert & de Backer, 2002a | Pigeon | 0.45 \pm 0.02 | 0.5 | 0.14 \pm 0.10 | 2.40 | 3.89 \pm 1.49 |
| Molter et al., 2013 | Hispaniolan Amazon Parrots | 0.285 | 1 | 0.232 \pm 0.22 | 15.9 | - |
| Lacasse et al., 2013 | Red-tailed Hawk | 1.4 | 0.5 | 0.832 \pm 0.711 | 0.78 | 0.38 \pm 0.37 |

Scientific names for the bird species: Chicken (*Gallus gallus domesticus*), Ostrich (*Struthio camelus*), Duck (*Anas platyrhynchos*), Turkey (*Meleagris gallopavo*), Pigeon (*Columba livia*), Hispaniolan Amazon parrots (*Amazona ventralis*), Red tailed hawks (*Buteo jamaicensis*), Great horned owls (*Bubo virginianus*) and African White-backed Vulture (*Gyps africanus*).

From analysing and reviewing published data on the pharmacokinetics of meloxicam in numerous species including mammals, it was evident that there was going to be species-specific variations of meloxicam in terms of its pharmacokinetic properties when comparing the data collected from this study with other avian species. However, there were a few discrepancies when analysing the results from this study with other chicken data. These differences in the pharmacokinetic parameters could be accounted for by several factors involved with the experimental design of the published data and the results from this study. This study used ISA Brown chickens which are used for egg production, whereas, the study conducted by Baert and de Backer in 2002 used broiler chickens which are used for meat production. The number of differences between the breeds are unknown but there are a few that may have influenced the results. The average weight of the chickens in the 2002 study were 2.2 ± 0.2 kg. As mentioned above, broiler chickens are produced for their meat and have been genetically selected for their higher muscle mass. As a result, this breed of chicken has a rapid growth rate and reaches slaughter weight at around 14 weeks old (Le Bihan-Duval, Berri, Baéza, Millet, & Beaumont, 2001; Le Bihan-Duval et al., 2008). Compare this to the ISA Brown chickens that were used in this study, which have been selected for their high egg production rate in terms of both quality of the eggs and duration in which they can sustain producing eggs. This results is a slower growth rate and are not slaughtered until their egg production slows down which is around 24 months of age (Hocking, Bain, Channing, Fleming, & Wilson, 2003). Two issues have been raised here: the average weight and the average age of the chickens in both studies. The weight of the broiler chickens in the 2002 study matched those chickens which were 24 months old ISA Brown chickens in

this study. Although the average the of the chickens in the 2002 study were not recorded, due to their different uses in the production industry, the broiler chickens would have been substantially younger than the 24 month old ISA Brown chickens regardless of the same average weight and possibly younger than the 18 week old ISA Brown chickens. If the variables of the animals were similar in their average weight and age in both studies and there were significant variations in the pharmacokinetic results, further investigation into the cause of the differences would be needed. So it is possible that differences between the breeds could have contributed to the variations seen when comparing the results between chickens.

Another difference between the studies that could have caused the variations in the results was the dose at which meloxicam was administered. In the 2002 study, the broiler chickens were given a single dose of meloxicam which was administered at 0.5 mg/kg (Baert & de Backer, 2002b), whereas, in this study the ISA Brown chickens has a single dose of meloxicam administered at 2 mg/kg. Prior to the commencement of this study, there was consultation with the veterinarians at the Wildbase Wildlife Health Centre Massey University, Palmerston North, about their use of meloxicam in various avian species that they commonly care for and their concerns about using meloxicam without the pharmacokinetic data to administer the correct dose and the dosing intervals. But from their anecdotal evidence within their clinic, they found that administering meloxicam at 2 mg/kg produced an effective analgesia in the various species without causing any major

side effects or harm to the patients. It was from this collaboration that this study decided to administer meloxicam at this dose. In addition, a study conducted by Naidoo and colleagues in 2008 used the same dose of meloxicam in six African White-back Vultures (*Gyps africanus*) without any side effects or causing any harm in the birds (Naidoo et al., 2008) therefore, there was published data on the safety of meloxicam being administered at this dose.

The methodologies also show some variation between the 2002 study and this study. For instance, the mobile phase in the 2002 study consisted of water-acetic acid: acetonitrile (65:35). The method chosen in this study replicated that in the study by Bae and colleagues, who determined the pharmacokinetics of meloxicam in human plasma (Bae et al., 2007). The mobile phase consisted of 20mM Potassium dihydrogen phosphate: acetonitrile (60:40) with a pH of 3.5. It was clear from the method validation phase of this study the formulation and ratio of the mobile phase and the pH of the solution had a clear impact on the retention of meloxicam in the High Performance Liquid Chromatography machine. The method used in this study provided the best retention of meloxicam in both the working standard and chicken plasma. As a result, the peaks of meloxicam in the chromatographs were well defined and isolated in order for the calculations of the pharmacokinetic parameters to be performed. In addition, the 2002 study analysed meloxicam under UV detector set at 305 nm, whereas, this study used a Diode Array Detector (DAD) which was set at 355 nm. The DAD is able to obtain more information over

a wider range of wavelengths, therefore, making it a lot more sensitive compared to a UV-detector.

Even though there were variations in the results that this study produced and published data, most of the pharmacokinetic parameters retained a similar trend with the other chicken data and also when compared to the other avian species. It is possible that the specific experimental designs of each study may have contributed to these variations.

The age of a chicken has shown to have significant influence upon several key pharmacokinetic parameters involved with the distribution, metabolism and elimination of meloxicam. From comparing the results between the 24 month old ISA Brown chickens and the 18 week old ISA Brown chickens, the mean residence time, volume distribution at a steady state, and the overall half-life showed to have significant differences between these two groups. The mean residence time (MRT) was significantly higher in the 24 month old ISA Brown chickens, therefore, the molecules of meloxicam remained in the 24 month old ISA Brown chickens longer in their system compared to the 18 week old ISA Brown chickens. From comparing the overall half-life ($t_{1/2}$) of meloxicam the result supports the significance found in the other parameters where it took longer for plasma concentration of meloxicam to decrease by half in the 24 month old ISA Brown chickens.

The examination of the volume distribution at a steady state (V_{ss}) of meloxicam shows that the 24 month old ISA Brown chickens have a significantly higher V_{ss} compared to the 18 week old ISA Brown chickens. This suggests that meloxicam had a higher binding affinity to the proteins in both the plasma and tissues in the 24 month old ISA Brown chickens compared to the 18 week old ISA Brown chickens. By meloxicam having a higher binding affinity and drug volume in the system it would support the higher MRT and the increase in the overall half-life ($t_{1/2}$) of meloxicam in the 24 month old ISA Brown chickens.

In this study, the metabolites of meloxicam were not measured therefore, we were unable to determine their influence upon the numerous pharmacokinetic parameters we measured. There are four inactive metabolites of meloxicam (refer to Section 2.1.3 on page 39 for details), which although they have no therapeutic effect still may influence the overall pharmacokinetic characteristics of meloxicam (Davies & Skjodt, 1999). In order to determine whether they do in fact influence the pharmacokinetics, a more sensitive method would be required to investigate this aspect, as the method used in this study was unable to detect any metabolites in the plasma samples. In the next chapter, more of the limitations and possible future work will be discussed in greater detail.

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Chapter Three

General Discussion

Painful experiences occur in many different ways resulting in a variety of experiences, intensities and durations for an individual (Johnson, 2002). The impact upon the animal's welfare mirrors the severity of the pain and whether the animal is suffering (Broom, 1991). The first step is the simple recognition that an animal is in pain, which is enough to advance the welfare of that individual. This acknowledgement enables the handlers and veterinary professionals to identify the source of pain and determine the course of treatment to treat the pain (Littler, 2007). It is because of this, decades of research has been carried out in order to understand the concepts and perception of pain (Johnson et al., 2005; Murrell & Johnson, 2006), part of which has included the development and efficacy of analgesics like NSAIDs to aid in improving the welfare of many animals.

Meloxicam pharmacokinetic studies that have been conducted in birds show a huge species-specific variation (Baert & de Backer, 2002a, 2002b; Lacasse et al., 2013; Molter et al., 2013; Naidoo et al., 2008). A study conducted by Baert and colleagues showed that meloxicam in chickens (*Gallus gallus domesticus*) and pigeons (*Columba livia*) which weighed between 0.45 and 2.2 kilograms, had a smaller volume of distribution compared to heavier birds. As a result, the clearance and elimination rate of meloxicam were faster in these lighter birds, regardless of a higher half-life (Baert & de Backer, 2002a). This assumption does not continue when other birds from other species are examined. For instance, birds in the same weight range like the Red-tailed Hawks (*Buteo jamaicensis*) and

Great Horned owls (*Bubo virginianus*) did not have the same relationship with the pharmacokinetic parameters. Instead, these birds had similar pharmacokinetic parameters to the ostriches (*Struthio camelus*) and turkeys (*Meleagris gallopavo*) who had an average weight of 19 and 8 kg respectively (Baert & de Backer, 2002a; Lacasse et al., 2013). Due to the lack of literature of pharmacokinetics of meloxicam in birds, no significant relationship has been found to determine why there is such a huge variation between avian species. It is obvious that there are still many factors involved in understanding the pharmacokinetics in birds which are unknown at this point.

The concept of age being an influencing factor upon pharmacokinetics of any analgesic drug has had little research done in humans, let alone in animals like birds. Most of the human studies that have used age as a discerning variable have not investigated the possible effects upon the pharmacokinetics of the drugs themselves. Instead, they examine the adverse effects NSAIDs may cause across the different age groups (Litalien & Jacqz-Aigrain, 2001; Musu et al., 2011; Smith & Baird, 2003; Titchen, Cranswick, & Beggs, 2005). There have been several studies investigating the effect upon the pharmacokinetics due to age by Bouwmeester and colleagues, which found significant age related changes in the pharmacokinetics of morphine of healthy neonates, infants and young children (Bouwmeester, Anderson, Tibboel, & Holford, 2004; Bouwmeester, van den Anker, Hop, Anand, & Tibboel, 2003). Although there was a significant difference between the ages, the differences in the pharmacology and pharmacokinetics of opioids makes it is hard to

draw direct conclusions to this study. Regardless of what analgesic drug was used, these studies do confirm that age is a variable which has a large influence upon pharmacokinetics in general, which gives this study a credible platform to launch an investigation into whether NSAIDs show a similar trend.

Although the area of aging birds and the associated effects that occur in older age is extremely limited in scientific literature, they show a similar decline in key organ functions and are more susceptible to diseases and conditions which affect their overall health, as in humans (Doneley, 2011; Holmes, 2004). In terms of a bird's life expectancy it depends on a diverse range of factors. Referring back to section 3.1 on page 65, table three depicts the vast differences that various avian species life expectancy. Such differences in a species' life expectancy depends on (but not limited to): their body mass, the larger the species, the longer they are expected to live (Mahmood, 2007); the age that they reach sexual maturity and reproductive rates; their purpose, whether that be as production animals, pets or conservation due to their endangered status; and the protective measures taken, for instance, wild birds do not have the access to veterinary intervention compared to birds kept as pets, thus impacting their life expectancy (Doneley, 2011). These factors further complicate the ability to understand and study aspects and the relationships which affect avian species and their veterinary treatment.

By researching the pharmacokinetics and pharmacodynamics of specific analgesic drugs, it enables specific and targeted treatments for a wider range of animals to be available for veterinarians. The majority of these studies have only been done in mammals, which limits the understanding of the interactions of the same drugs in other species like avian species. As a result, dosing information for analgesic drugs such as meloxicam for birds is often procured through the extrapolation of mammalian data. The process of allometric scaling determines the relationship between the basal metabolic rate (BMR) and the animals' body mass. The result of collating the relationship between these two variables with numerous species creates a linear relationship which can be used to further extrapolate data. In the field of pharmacokinetics, if an analgesic drug has not been studied in a specific animal, the data from allometric scaling can be used to determine the correct dosage per kilogram for that animal, with the assumption that their anatomy, physiology and biochemistry are relatively similar (Hunter & Isaza, 2008).

A major issue arises when the dosage for a bird is required due to its different anatomy, physiology and biochemistry. They differ extensively from mammals, therefore, the relationship between avian species BMR and body mass creates a different linear relationship to that of mammals. So extrapolating dose information from the mammalian data set is often incorrect, which can cause a number of issues for the bird, especially when dealing with any kind of drug. From the limited literature present on allometric scaling in avian species, fewer errors occur when extrapolating data from only avian

species when compared to extrapolating from mammalian data (Hunter & Isaza, 2008; Hunter et al., 2008). In determining the pharmacokinetics of a particular drug in a certain avian species, it expands the data pool available for other avian species that that may be unavailable for such research, like rare or exotic birds. The objectives for this research was to conduct a pharmacokinetic study of meloxicam in ISA Brown chickens, where it explored whether the age of a chicken had an effect on the pharmacokinetics. By investigating the basic pharmacokinetic parameters of meloxicam in the ISA Brown chickens the results from this study will contribute to the set of bird-specific pharmacokinetic information, which can be used to extrapolate dosage for other birds. Furthermore, the investigation into the possible effects of age upon the pharmacokinetics of meloxicam will deepen our understanding on how other variables may possibly influence how we calculate doses that are effective but limit the negative side effects or outcomes that arise due to the lack of information available.

Conclusion

By comparing the meloxicam pharmacokinetic data from this study and the other similar studies (Baert & de Backer, 2002a, 2002b; Lacasse et al., 2013; Molter et al., 2013; Naidoo et al., 2008), chickens had a lower volume of distribution and the clearance rate of meloxicam was slower, compared to other avian species. Furthermore, the half-life of meloxicam in chickens was significantly longer than most of the other species. The only species that had a similar half-life was the Hispaniolan Amazon parrot after a single 1

mg/kg intravenous dose (Molter et al., 2013). As a result of the increased half-life, both the mean residence time (MRT) and area under the curve (AUC) is considerably higher in the chickens when compared to other avian species.

To investigate whether the age of a chicken influenced the pharmacokinetics of meloxicam, this study used a group of 10 ISA Brown chickens that were 18 weeks old and a second group of 10 ISA Brown chickens that were 24 months old. The age of a chicken has shown to have significant influence upon several key pharmacokinetic parameters involved with the distribution, metabolism and elimination of meloxicam. The elimination half-life, steady state volume of distribution and mean resident time were significantly higher in the 24 month old ISA Brown chickens compared to the 18 week old ISA Brown chickens. Overall, the results indicate that as an ISA Brown chicken ages the pharmacokinetics of meloxicam show some significant changes in crucial pharmacokinetic parameters. The mean residence time (MRT) was significantly higher in the 24 month old ISA Brown chickens, this suggests that the molecules of meloxicam remained in the 24 month old ISA Brown chickens in their system longer compared to the 18 week old ISA Brown chickens. From comparing the overall half-life ($t_{1/2}$) of meloxicam the result supports the significance found in the other parameters where it took longer for plasma concentration of meloxicam to decrease by half in the 24 month old ISA Brown chickens.

The examination of the volume distribution at a steady state (V_{ss}) of meloxicam shows that the 24 month old ISA Brown chickens have a significantly higher V_{ss} compared to the 18 week old ISA Brown chickens. This suggests that meloxicam had a higher binding affinity to the proteins in both the plasma and tissues in the 24 month old ISA Brown chickens compared to the 18 week old ISA Brown chickens. By meloxicam having a higher binding affinity and drug volume in the system it would support the higher MRT and the increase in the overall half-life ($t_{1/2}$) of meloxicam in the 24 month old ISA Brown chickens.

In conclusion, the pharmacokinetics of meloxicam in ISA Brown chickens after a single intravenous dose of 2 mg/kg varies with age as observed in other published data on humans. By comparing the age of the ISA Brown chickens and their associated pharmacokinetic data, there were several differences between several key pharmacokinetic parameters which suggests the age of a bird is a variable which needs to be considered and studied further in order to safely calculate the correct dose of meloxicam. In turn, promoting better healthcare treatment for the individual and the overall welfare concerns that arise with inadequate pain control for animals.

Limitations

As mentioned earlier in this section, there is very little scientific literature investigating whether age is a significant influence upon pharmacokinetics even in human. Most of the research surrounding this topic is focused on the pharmacodynamic changes seen as a human ages (Bateman & Kennedy, 1995; Litalien & Jacqz-Aigrain, 2001; Musu et al., 2011; Smith & Baird, 2003; Titchen et al., 2005). As a result, this makes it hard to determine whether or not the results from this study are truly significant in terms of the overall impact that age may have upon the pharmacokinetics in any avian species. Although this study showed there were significant difference between numerous pharmacokinetic parameters, there is no pre-existing relationship between the age of an individual and pharmacokinetic changes of NSAIDs in any avian species that these results can be compared to. The only evidence available to support the results from this study come from a very early study by Woolf and colleagues in 1983, who looked at whether the pharmacokinetics of piroxicam differed due to age. Although they concluded that there was no conclusive evidence that the half-life, clearance rate or volume of distribution showed any difference between the different ages in this particular study, it was not until they compared their results with another study of piroxicam in young healthy adults that was conducted by Rodgers and colleagues, there was evidence to suggest that there was a significance difference between the two age groups in the pharmacokinetics of piroxicam (Woolf et al., 1983). But due to advancement in pharmacokinetic techniques and

methods, comparing the results from Woolf's study and the results from this study should be done with a large amount of scepticism. Other than this evidence, there is no current literature investigating these exact variables in any animal to compare our results with.

In the pharmacokinetic studies of meloxicam that have been conducted with avian species, the age of the birds was not mentioned or taken into consideration when completing their studies. Therefore, the conclusions made in this study against the current data available may also have discrepancies. For instance, the differences between species may not be so pronounced if all the ages of the birds were exactly the same, which is clearly impossible. As a result, it is hard to conclude that the differences seen in the pharmacokinetic parameters are species-specific differences or whether other variables like age, influence these differences.

As mentioned before, the 24 month old ISA Brown chickens that were used in this study are considered to be 'old' for poultry because of their decline in productivity levels rather than their actual age. By 24 months, their overall productivity levels decline along with the nutritional components within the eggs themselves (Grobas, Mendez, De Blas, & Mateos, 1999). For example, by the age of 17 months the egg white volume has increased, however the percentage of albumin within the whites has dramatically declined compared to 22 week old hens (Grobas et al., 1999). Once the hens have reached this 'old' age, they are culled in order for the industry to maintain productivity levels.

In comparison, there are numerous avian species that can live anywhere between 10 to 60 years, like budgerigars and various parrot species (Doneley, 2011). These long-lived species are often kept as pets or are protected in sanctuaries, where veterinary intervention can easily prolong the lives of these birds. It is these birds which are more likely to exhibit age related changes in their organ functions and are more likely to show changes in the pharmacokinetics of numerous drugs like meloxicam. Therefore, to accurately determine whether age influences the pharmacokinetics of meloxicam, studies should be conducted in these long-lived species, who are likely to die from diseases or complications associated with their age. But these studies have both ethical and logistical issues that would hinder this kind of research. For example, sourcing and obtaining the same species and at such an old age would be very difficult, along with the welfare considerations that must be taken into account when dealing with fragile and possibly rare bird species.

In this study, the ISA Brown chickens were used to model other avian species due to already being a prominent and established model species in current literature, with also their accessibility, and low cost, and we were able to obtain the two different age groups more easily due to the need for the poultry farm to continually replenish their stock. More importantly, there are ethical constraints when experimenting with animals and by using the chickens as a model it ensures the protection of other species which may be at risk

from such experimentation. Initially, this study was to cover three of New Zealand's native bird species. The birds as intended subjects were already at the Wildbase Wildlife Health Centre as in patients for treatments unrelated to this study but part of their treatment was the use of meloxicam as an analgesic. To protect these birds, qualified veterinarians would have been required to obtain the blood samples in order to reduce the stress to the birds and for the required protocols to be followed, all of which would have protected their welfare and the ethical components required during this experimentation phase of the study.

In this study, the concept of population pharmacokinetics were used, where the overall pharmacokinetic differences and efficacy of a drug are analysed within a subgroup/s of a population. More importantly, we used the standard approach of using numerous samples from a select number of individuals. This involved obtaining the pharmacokinetic parameters of an individual and then combining to obtain the overall mean and variability of the pharmacokinetic parameters within that subgroup of the population (Riviere, 2011b). By ignoring the individual's metabolism, biochemistry, rate of absorption and elimination the variability of the individual's response to meloxicam was ignored. Instead, this approach gave an overview of the response to meloxicam for the two different age groups. If the individual differences were examined, there would have been a huge variation seen regardless of their age. In addition, it would not provide us with any useful information about the overall pharmacokinetic response and efficacy of

meloxicam in birds. However, it could be argued that the differences that were seen, could actually be the result of the variation being so great between individuals, that the overall pharmacokinetic parameters were significantly different between the two ages. Due to the lack of literature on age related changes in pharmacokinetics, it is hard to determine whether it was individual variation that contributed to the differences or if age does have an effect on the pharmacokinetics of meloxicam.

Future work

This study was aimed at increasing the general knowledge about the pharmacokinetics in birds by using chickens as a model. The pharmacokinetic results recorded from these chickens, regardless of their age, are similar to those found in similar studies (Baert & de Backer, 2002a, 2002b). This study also found that the pharmacokinetics of meloxicam differed between the 18 week old ISA Brown chickens and the 24 month old ISA Brown chickens. One attribute we did not investigate in this study was the metabolites of meloxicam and whether the presence of the metabolites influenced the overall pharmacokinetic properties of meloxicam in this species. So this aspect should be included in any future work investigating meloxicam pharmacokinetics. Conducting pharmacodynamic studies in chickens with a similar age difference, may further support the findings in this study, as well as improving the efficacy of meloxicam as an analgesic drug.

Further studies are needed to look into the mechanisms behind the pharmacokinetics of meloxicam but also other NASIDs in birds. This will impact significantly on understanding the differences between drugs and how they act in relation to the anatomical, physiological and metabolic differences of mammals and birds. In this study we used a breed of chicken which is used in the production industry for their egg production. During the experimental phase of this study the question was raised about whether the administration of meloxicam could cause the metabolites to be transferred into the unfertilised eggs during development within the hen's reproductive system. As a precaution, all the eggs that were produced from the chickens after the administration of meloxicam were considered not to be available for human consumption and were thrown away. In the production industry, it is unlikely that the chickens would ever be administered drugs like meloxicam so there would be no issue in the possibility of the transmission of meloxicam. However, it may be an avenue to investigate the possibility for this transmission to occur in other species which are classified as pets and their eggs are procured for human consumption, or are used for breeding purposes. If there is transmission of meloxicam into the egg, we could use similar principles when analysing the time taken for meloxicam to become undetectable in plasma, in the egg contents.

By gathering more information, other techniques for administering such drugs like meloxicam can be investigated and improved. For instance, developing a subcutaneous

emulsion of meloxicam that can be injected into birds but has a similar function as the long-acting NSAID transdermal patches used on mammals today may further reduce the possible side effects while maintaining a longer mean effective concentration (MEC) of the drug.

As mentioned in the limitations section, the initial study intended to determine the pharmacokinetics for both meloxicam and the opioid butorphanol in three New Zealand native species: the Kiwi, Kereru and the New Zealand Harrier. One of the main goals for this study was to determine the pharmacokinetics of each drug so that the veterinarians at the Wildbase Wildlife Health Centre could directly use the results found in the study when administering either drug in their patients. More importantly, the results would have been completely tailored to each species that was involved in the study. This would have allowed the veterinarians to determine the dose and dosing intervals for either meloxicam or butorphanol for a specific species. It was the veterinarian's frustration with the lack of pharmacokinetic scientific literature in these two drugs which prompted this study to be designed in the first place. Due to time constraints, another study had to be designed in place of this study. Regardless of the logistical issues that occurred in this study, it is still an area which still needs investigation into plus by tailoring the study to these specific species it contributes the conservation efforts that are being put into these three New Zealand native species.

This present study also ran into several unforeseen problems which resulted in the study only focusing on whether the age of ISA Brown chickens affected the pharmacokinetics of meloxicam. Like the initial study, this study was originally presented to include the opioid drug butorphanol. The basic pharmacology of NSAIDs and opioids differ significantly in terms of their action sites within the body. As mentioned in the first chapter, NSAIDs inhibit the production of COX-1 and COX-2 which are the precursors to the production of prostaglandins. Whereas, opioid drugs like butorphanol target specific opioid receptors predominantly distributed within the central nervous system, particularly in the areas which belong to the descending pain pathways (Trescot et al., 2008). These receptors become activated when they bind with exogenous opioids and cause the inhibition of neurotransmitters. This prevents the further transmission of pain signals within the central and peripheral nervous system (Trescot et al., 2008). If we had the pharmacokinetic data for both drugs it would have given us the ability to compare their basic pharmacokinetic properties against each other and whether there were any differences due to age in either drug. It is evident that there is severe lack in pharmacokinetic data in avian species in any drug/s. If we were able to include butorphanol in this study, it would have enabled us to contribute more information and fill in more of the missing pieces that surround this vast gap in the literature. Therefore, it would be another avenue to explore in future studies.

Overall, any future work in this area surrounding the pharmacokinetics of various drugs in avian species will greatly increase the general knowledge we severely lack at this

time. All of which will feed into the ability to produce avian specific allometric scales where the calculations of drugs will increase in their accuracy and safety for many avian species requiring drug therapies and expanding the information regarding other variables like age which may impact the pharmacokinetics of a drug. All of this will ultimately help treat this group of animals, especially as they are becoming more popular as pets but also the list of endangered avian species is continuing to grow and by understanding and tailoring pharmacokinetic information specifically to birds aids the conservation efforts that are being undertaken to protect these species in order for them to survive.

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