THE EFFECT OF DIETARY CADMIUM ON KIDNEY FUNCTION IN CATS

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Abstract

Due to the requirement for meat in feline diets, this study aimed to investigate the potential effects on kidney function in cats of cadmium accumulation in meat products due to pasture management practices. Cadmium may be a causal factor in feline Chronic Kidney Disease (CKD). Twenty-seven domestic short hair cats were randomly selected from the colony population of the Feline Nutrition Unit of Massey University and assigned to three experimental groups (n=9), which were balanced for age and sex. Each group received one of the three experimental diets designed to represent the full range of potential cadmium concentrations that cats may be exposed to on wet diets in New Zealand. Diets were fed *ad libitum* for a 6-month period. Kidney function was examined at baseline and after 3 and 6 months by measuring glomerular filtration rate (GFR) using iohexol clearance analysed by high performance liquid chromatography (HPLC). Blood and urine analyses were also conducted on a monthly basis. While GFR fluctuated over the study period no significant differences were found either between groups at the end, or within each group when compared at the beginning and end of the study. Although overall no evidence of CKD was observed, an unexplained trend of weight loss was observed in females receiving the two diets containing the highest cadmium levels, which may simply have reflected reduced dietary palatability. The results of the study showed no detectable effects of feeding the three diets for 6 months; however, an extended trial period may be required to fully investigate the longer term effects of cadmium levels and other dietary factors on the development of CKD. In particular, more work is needed to explore the potential for genetic and/or functional differences in mechanisms which are involved in the transport, and/or deposition of cadmium, or are protective against cadmium toxicity in cats and to further define normal parameters and standard approaches in measuring GFR in cats.

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List of Abbreviations

BW   Body Weight

CBC   Complete Blood Count

Cd   Cadmium

CKD   Chronic Kidney Disease

DM   Dry Matter

GFR   Glomerular Filtration Rate

HPLC   High Performance Liquid Chromatography

HR   Heart Rate

IRIS   International Renal Interest Society

MT   Metallothionein

PTWI   Provisional Tolerable Weekly Intake

SBP   Systolic Blood Pressure

USG   Urine Specific Gravity

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Chapter 1   Introduction

New Zealand has the highest rate of pet ownership in the world, with 68% of New Zealand households owning some kind of pet, with the total number of pets outnumbering the human population (New Zealand Companion Animal Council, 2011). Cat ownership (again the highest in the world *per capita*) represents 48% of all households; 28% of households own one cat and 20% own two or more cats (New Zealand Companion Animal Council, 2011).

Feline chronic kidney disease (CKD) is a common disease affecting cats (Cobrin et al., 2013; Paepe & Daminet, 2013), particularly older/geriatric cats (Elliott & Barber, 1998; Chakrabarti et al., 2012). The overall prevalence of CKD is thought to range from 1.6-20% in cats (Lund et al., 1999; Polzin, 2010), rising to up to 32% in cats aged over 15 years (Polzin, 2010). CKD is an irreversible loss of renal function and/or structure that remains stable for some period of time but is ultimately progressive (Bartges, 2012). CKD occurs when compensatory mechanisms are no longer able to maintain the excretory, regulatory, and endocrine functions in animals with kidney disease (Nelson & Couto, 2014). For cats with CKD timely diagnosis and staging can facilitate the initiation of medical management improving the prognosis (Paepe & Daminet, 2013).

Exposure to cadmium (Cd) has been implicated in the development of CKD in humans (Templeton & Chaitu, 1990; Dorian et al., 1992; Uriu et al., 1998; Sigel et al., 2013), however little evidence is available on how cats may be exposed to cadmium (Finch et al., 2012), the cadmium levels in the organs of cats (Paßlack et al., 2014), and how this relates to potential pathologies, particularly of the kidneys (Andreani et al., 2010; Finch et al., 2012; Paßlack et al., 2014). It is important to evaluate if the accumulation of cadmium is dependent on kidney function and in particular, disorders in the renal excretory mechanisms, and whether these may either increase or decrease cadmium deposition in the kidneys of cats (Paßlack et al., 2014).

Higher cadmium concentrations (in the liver and renal cortex) have been found post mortem in cats with CKD compared to healthy animals (Paßlack et al., 2014). Cadmium concentrations in the liver and renal cortex were also age dependent, with lower concentrations in cats aged 0-7 years compared to those 12-18 years (Andreani et al., 2010; Paßlack et al., 2014).

Cadmium is an elemental impurity that is naturally present in phosphate rock, and consequently, in all phosphate fertilisers (Cadmium Working Group, 2008; Stafford et al., 2014). Phosphate fertilisers are a critical component in increasing productivity in New Zealand agriculture (Cavanagh et al., 2013), particularly in the dairy sector (Cavanagh et al., 2013). In New Zealand, pastoral (average 2.70µg/g) and horticultural (average 0.50µg/g) land-use has resulted in soil concentrations of cadmium that are much higher than the national average (0.35µg/g), with accumulation of cadmium in these soils indicative of high fertiliser use (Taylor, 2007). As a consequence of the application of mineral fertilisers in particular (He et al., 2005), cadmium has been detected in processed meats (Duran et al., 2010). When grown on a cadmium-polluted soil, some crops, such as rice, can accumulate substantial amounts of cadmium (>1000 µg/kg) (World Health Organization Regional Office for Europe, 2000). Animals grazing on fertilised lands have the potential to incorporate high cadmium levels in their tissues (Paßlack et al., 2014). The highest cadmium levels have been found in mammalian organ meats, particularly kidney and liver (World Health Organization Regional Office for Europe, 2000).

In New Zealand, kidneys from older cattle and sheep are excluded from human consumption due to the possibility of elevated cadmium levels (Cavanagh et al., 2013; Ministry for Primary Industries, 2013; Stafford et al., 2014). However these kidneys can be diverted for use in pet foods (usually wet cat or dog food) or meat and bone meal production on the basis that kidneys would be subject to significant dilution in the final products (Ministry for Primary Industries, 2013).

New Zealanders spend approximately $1.584 billion a year on their companion animals with the biggest areas of expenditure being pet food, veterinary care and healthcare products. Pet food accounts for 48% of all expenditure, with an estimated total spend of $766 million a year. In 2010 owners spent $235.7 million on cat food, up 0.6% on the previous year. Wet cat food accounted for 63% of sales, with 35% dry cat food and the remaining 2% treats and mixers (New Zealand Companion Animal Council, 2011). The consumption of such large amounts of wet food by cats in New Zealand may result in exposure to significant levels of cadmium (New Zealand Companion Animal Council, 2011).

This thesis will therefore present work investigating the effects of feeding high levels of cadmium in cat food on renal function in cats when compared to diets with lower levels of cadmium. The work is presented as 5 chapters; Chapter 2 reviews the relevant literature regarding cadmium as a possible contributor to Chronic Kidney Disease (CKD) in cats, Chapter 3 discusses the chosen methodology and study design, Chapter 4 reports the results, Chapter 5 discusses the results in the context of the literature and the existing knowledge and draws final conclusions.

Chapter 2 Literature Review

2.1 Nutritional requirements of cats

2.1.1 Nutrient requirements

Domestication has changed cats relatively little. Of all domesticated animals, they retain the most anatomic, metabolic, and behavioural features of their predecessors (Bradshaw, 2006; Zoran & Buffington, 2011). Cats are obligate carnivores which means that in their natural habitat, cats consume prey and rely on ingestion of nutrients from animal tissues (Verbrugghe & Bakovic, 2013). Due to evolutionary pressure it is thought that particular key metabolic enzymes have been lost in a common ancestor of all of the existing species in the cat family (Morris, 2002; Bradshaw, 2006). As a result cats have developed several physiological and metabolic adaptations, including a number of idiosyncrasies in protein, carbon, and fatty acid metabolism that have led to specific and unique nutritional requirements for essential nutrients from animal based ingredients, such as arachidonic acid, vitamins (including A, D and B - particularly niacin), and the amino acids taurine and arginine (MacDonald et al., 1984; Morris, 2002; Zoran, 2002; Thompson, 2008; Eisert, 2011; Verbrugghe et al., 2012). These nutrients either cannot be endogenously synthesised in sufficient amounts to meet their needs or cannot be synthesised at all (MacDonald et al., 1984; Morris, 2002).

It is however, their unique need for large amounts of dietary protein (specifically, dispensable nitrogen) that separates cats from non-carnivorous domestic species (MacDonald et al., 1984; Morris, 2002; Eisert, 2011). Recent analyses of gluconeogenic capacity suggest that cats do not have a high protein requirement *per se*, rather that they have a secondarily elevated protein requirement in response to a high endogenous glucose demand (Eisert, 2011; Verbrugghe et al., 2012). Higher dietary intake of protein in cats compared to other species (about 2 to 3 times higher in cats than other adult non-carnivores) (Morris, 2002; Verbrugghe & Bakovic, 2013) is dictated by an accelerated use of amino acids, coupled with an inability to conserve amino acids such as methionine, cysteine, taurine and arginine (Verbrugghe & Bakovic, 2013). However, while cats have some ability to adjust the catabolism of amino acids from lower intakes of protein (Verbrugghe & Bakovic, 2013; Wester et al., 2015), it is not clear why they cannot adjust to levels which are sufficient for other species (Verbrugghe & Bakovic, 2013).

2.1.2 Requirement for meat in feline diets

Vitamin A and taurine are just two examples of the essentiality of meat in the feline diet. Cats cannot convert β-carotene (which is plentiful in plants and is hence present in some commercial pet foods) to retinol (the active form of vitamin A) (Gershoff et al., 1957) because they lack the necessary intestinal enzyme 15, 15'-dioxygenase (Morris, 2002; Zoran, 2002). As a result, in feline diets vitamin A must be provided in the biologically active form which is found naturally only in animal tissues (Gershoff et al., 1957).

Taurine, the most abundant free amino acid in animal tissues (Verbrugghe & Bakovic, 2013), is also only naturally present in foods of animal origin (Sturgess & Hurley, 2005). Taurine is found in high concentrations in heart, brain, muscle and retinal tissues and is linked to physiological functions such as osmoregulation, calcium modulation, anti-oxidation, and neurotransmission (Hamper et al., 2012; Froger et al., 2014). Unlike dogs, cats have an absolute requirement for taurine as they are unable to synthesise sufficient taurine from methionine and cysteine to meet their needs, due to reduced activity of two enzymes; cysteine dioxygenase (which catalyses the oxidation of cysteine to cysteinesulphinic acid) and cysteine sulphinic acid decarboxylase (which catalyses the conversion of cysteinesulphinic acid to hypotaurine) (Morris, 2002; Sturgess & Hurley, 2005). The low affinity by these enzymes has a multiplicative effect meaning traffic along the pathway becomes insignificant (Morris, 2002). Further exacerbating the situation is the fact that cats have an increased requirement for taurine to manufacture conjugated bile salts in order to emulsify dietary fats prior to digestion and absorption, while other species can use taurine or glycine (Sturgess & Hurley, 2005).

While meat is important in the feline diet, it is important to balance the types of meat, for example lean meat versus offal. Lean meat contains excessive amounts of phosphorus relative to calcium and is deficient in sodium, iron, copper, iodine and vitamins. An all-lean meat diet can thus lead to severe and potentially fatal skeletal abnormalities, joint malformations, essential fatty acid deficiencies, and nutritional secondary hyperparathyroidism (Sturgess & Hurley, 2005). Organ meats, such as liver, contain high levels of vitamin A, which in excess leads to painful bone deformities including, ankylosis (stiffening and immobility or fusion of a joint), hyperostosis (excessive/abnormal thickening or growth of bone tissue) or exostosis (a bony growth on the surface of a bone). These conditions do not resolve, even if the diet is corrected (Seawright & Hrdlicka, 1974; Bennett, 1976; Sturgess & Hurley, 2005; Bartges et al., 2012).

Small felids that subsist primarily on a diet of rodents will eat their prey whole, organs included. Large cats are thought to preferentially eat organ meats because they represent a rapid source of nourishment; organ meats have a high nutrient density, favourable nutrient profile (containing high levels of the amino acids methionine and taurine) and high digestibility (Aldrich, 2012). Methionine is present in liver and kidney in higher levels (1.65% and 1.53% respectively) on a DM basis, than lean meat (1.05%) (National Research Council, 2006), with the upper limit in cat foods 1.5% on a DM basis (AAFCO, 2015). Furthermore, considering taurine, liver contains much higher levels of taurine (2.23% DM) than kidney (0.1% DM), and lean meat (0.16% DM) (National Research Council, 2006), and contains significantly more than the minimum requirements (0.2% in canned cat food) (AAFCO, 2015).

Proteins ingested from the diet are hydrolysed in the stomach and intestine to amino acids. Amino acids excess to immediate requirements are deaminated from amino nitrogen and result in the toxic waste product ammonia which is further processed into urea (Figure 2.1). The production of urea is essential in preventing an accumulation of ammonia. The amino nitrogen resulting from amino acid catabolism is used in the first step of the urea cycle (Figure 2.2) for the conversion of ornithine (Elliott & Elliott, 2009), the precursor of arginine (Morris & Rogers, 1982).
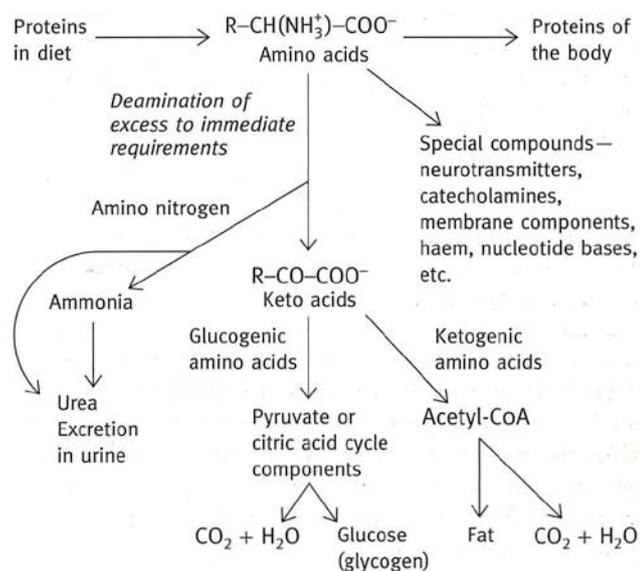
Figure 2.1 - The overall catabolism of amino acids (Elliott & Elliott, 2009).
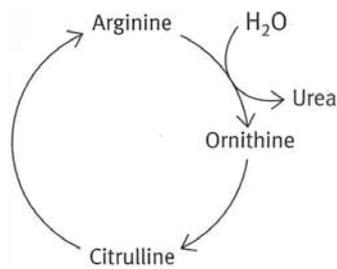
Figure 2.2 - Outline of the arginine- urea cycle (Elliott & Elliott, 2009).

While the liver is recognised as the primary site of ureagenesis, there is evidence to suggest the kidney is also key in producing the arginine required by the peripheral tissues (Morris & Rogers, 1982). When given a diet devoid of arginine, cats are unable to synthesise sufficient ornithine to maintain optimal activity of the urea cycle essential in preventing hyper-ammonaemia (Morris & Rogers, 1982).

2.2   Kidney structure and function

The kidney is generally considered to have three distinct regions (see Figure 2.3), which can be visualised by the naked eye. The renal cortex forms the outer region and is lighter coloured; deeper toward the centre of the kidney (a darker red-brown area) is the renal medulla; and within the medulla, the renal (or medullary) pyramids. These are many roughly triangular regions with a striped appearance. The base of each pyramid faces toward the cortex while the apex (tip) points towards the inner region of the kidney. The structural and functional units of the kidneys are nephrons, which are responsible for forming urine; there are over a million per kidney that feed into collecting ducts, which take fluid from several nephrons and convey it to the renal pelvis (Marieb, 2015).
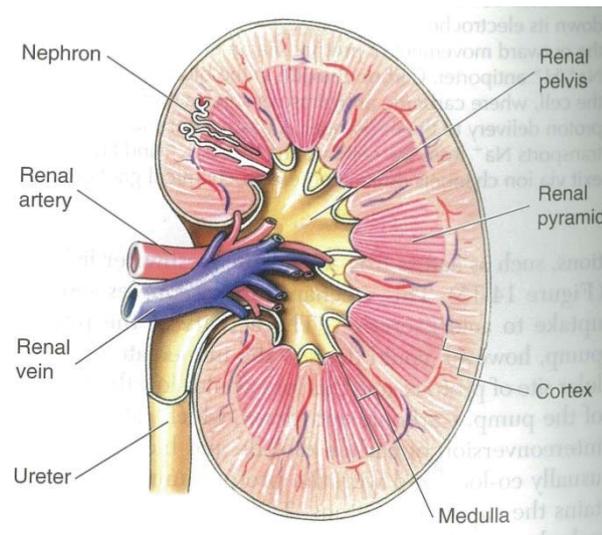
Figure 2.3 - Cross section of a kidney. From: (Randall et al., 2002), figure 14-17, Copyright 2002 by W.H. Freeman and Company. (Used by Permission of the publisher).

A nephron itself is made up of two main structures: a renal corpuscle and a renal tubule. The renal corpuscle consists of a glomerulus (a knot of capillaries), and the glomerular (or Bowman's) capsule, a cup-shaped, hollow structure that completely surrounds the glomerulus. The inner layer of the capsule is made up of highly modified cells called podocytes which have long branching processes (extensions) that intertwine with one another and cling to the glomerulus. Filtration slits (openings) exist between podocyte extensions forming a porous membrane around the glomerulus (Marieb, 2015). The renal tubule, which makes up the rest of the nephron, extends from the glomerular capsule where it coils and twists, forms the loop of Henle, before again becoming coiled and twisted before entering a collecting duct (Marieb, 2015) as shown in Figure 2.4 below.

Structure of the nephron

Proximal tubule

Afferent and efferent arterioles

Cortex

Bowman's capsule

Glomerulus

Medulla

Distal tubule

Collecting duct

Loop of Henle

Figure 1 Ultrafiltrate filtered from the glomerulus of the nephron enters the Bowman's space within the Bowman's capsule, then passes through the proximal tubule, loop of Henle, distal tubule and collecting duct to form urine

Structure of the glomerulus

Afferent arteriole

Glomerular ultrafiltrate in Bowman's space

Efferent arteriole

Glomerular (Bowman's) capsule

Figure 2 The glomerulus – a network of capillaries interposed between the afferent and efferent arterioles – is enclosed within an epithelial capsule (Bowman's capsule) and separated by the Bowman's space.

Glomerular filtration is a pressure-driven process governed by hydrostatic and oncotic pressures in the glomerular capillaries and Bowman's capsule, and by glomerular conductivity and the capillary surface area

Figure 2.4 – Structure of the nephron and glomerulus (Finch, 2014) (Reproduced by permission of author and right's holder).

Most nephrons are called cortical nephrons because they are located almost entirely within the cortex and are short looped (Marieb, 2015). A few nephrons are called juxtamedullary nephrons because they are located near the boundary of the cortex and medulla, and their loops penetrate deep into the medulla (Akers, 2013). Each collecting duct receives urine from many nephrons and run downwards through the medullary pyramids delivering the final urine product into the calyces and renal pelvis (Marieb, 2015).

The peritubular capillaries emerge from the efferent arteriole that drains the glomerulus and adhere closely to the length of the renal tubule. Unlike the glomerulus, these capillaries are low-pressure, porous, and adapted for absorption instead of filtration. As filtrate (essentially blood plasma without blood proteins) circulates through the renal tubule, peritubular capillaries receive reabsorbed solutes and water from the tubule cells and ultimately drain into interlobar veins leaving the cortex (Marieb, 2015).

Glomerular filtration is a nonselective, passive process where fluid (filtrate) passes from the blood into the glomerular capsule. Both proteins and blood cells are normally too large to pass through the membrane, and indicate a problem with glomerular filtration if either of these appears in urine. Filtrate is formed as long as the systemic blood pressure is normal; if arterial blood pressure drops too low, glomerular pressure becomes inadequate to force substances out of the blood into the tubules, and filtrate formation stops (Marieb, 2015).

Besides wastes and excess ions that must be removed from the blood, filtrate contains many useful substances (such as water, glucose, amino acids, and ions), which need to be reabsorbed and returned to the blood. Tubular reabsorption begins as soon as the filtrate enters the proximal convoluted tubule. The tubule cells act as transporters taking up required substances from the filtrate and are then absorbed into blood at the peritubular capillaries. While some reabsorption is passive (such as water by osmosis), most reabsorption depends on active transport via selective membrane carriers (Marieb, 2015). There is an abundance of carriers for substances that need to be retained (such as glucose and amino acids), and few or no carriers for substances of little or no use to the body including the nitrogenous waste products urea (formed by the liver as an end product of protein breakdown when amino acids are used for energy), uric acid (released by nucleic acid metabolism), and creatinine associated with creatine metabolism in muscle tissue. Substances required by the body are usually completely removed from the filtrate, meanwhile because tubule cells have few membrane carriers to reabsorb the 'less desirable' substances these are poorly reabsorbed tending to remain in the filtrate and therefore found in high concentrations in urine (Marieb, 2015).

Various ions are reabsorbed or excreted in the urine, depending on what is needed to maintain the appropriate pH and electrolyte balance of the blood at a particular time. Most reabsorption occurs in the proximal convoluted tubules, but the distal convoluted tubule and the collecting duct also contribute. Tubular secretion on the other hand is essentially the reverse of tubular reabsorption. Some substances, such as hydrogen and potassium ions (H^+ and K^+) and creatinine, move from the blood of the peritubular capillaries through or from the tubule cells into the filtrate to be eliminated in urine. This process is important for getting rid of substances, such as certain xenobiotics, excess potassium, or as an additional way of controlling blood pH (Marieb, 2015). The loss of these solutes (particularly urea and sodium) leads to a reduced solute gradient (medullary hypertonicity), which impairs the ability of the nephron to produce concentrated urine (Lunn & James, 2007).

2.3   Urine Concentrating Mechanisms

Most mammals produce concentrated urine, a process that lies at the centre of the kidney's role in fluid and solute homeostasis. Despite decades of research, the production of the corticomedullary osmotic gradient, much of which is a passive process that underlies the concentrating process, remains largely unexplained (Pannabecker, 2013).

Current understanding of urine concentration is that counter-current exchange, due to the counter-flow arrangement of descending and ascending vasa recta, traps sodium chloride (NaCl), and urea is deposited to the interstitium by collecting ducts and the loops of Henle (Pannabecker, 2013). The formation of concentrated urine results from the structure of the nephron and vasa recta capillaries, and additionally specific transport properties (NaCl, urea, water) of individual nephron segments (Kriz & Kaissling, 2000).

There is a continuous obligatory water loss via the kidneys, even under extreme dehydration. The ability of kidneys to conserve water is related to their concentrating capacity; the more concentrated the urine, the less water is excreted with a given amount of solute (Anderson, 1982). The generation of concentrated urine is related to the presence of a fully developed medulla. While all mammalian kidneys possess a renal cortex, rodents without an inner medulla, like the beaver, are unable to generate concentrated urine (Bankir & de Rouffignac, 1985). Renal medullary thickness has been used as an index of an organism's ability to generate concentrated urine and subsequently linked as an adaptation to dry habitats; however these kidney indices must be used with caution (Bozinovic & Gallardo, 2006).

Several reviews have analysed reported kidney indices (Bozinovic & Gallardo, 2006), however they are unable to explain what occurs physiologically when the maximal urine osmolality does not fit the index. This index does not take into allow for the structural organisation of tubules and blood vessels in the medulla. It is often positively correlated with the proportion of long looped nephrons, which have successively been positively correlated with urine concentrating ability. In rodents with high urine concentrating abilities, such as desert-adapted Sand rats (*Psammomys spp*); short looped nephrons outnumber long looped nephrons (Kriz & Kaissling, 2000). In contrast, the kidneys of cats and dogs have been described as having predominantly long looped nephrons (Bozinovic & Gallardo, 2006). In comparison with other species, the ability of the cat to concentrate urine is 25% greater than the dog (Anderson, 1982), slightly higher than Merino sheep (a semi-arid adapted breed of sheep) (Macfarlane et al., 1958; Anderson, 1982) and a little more than half of the maximum values of some desert-living rodents (Anderson, 1982). Medullary thickness and the number of long looped nephrons are

therefore not always linked to the generation of highly concentrated urine (Bozinovic & Gallardo, 2006).

Of relevance to the arguments regarding urine concentrating mechanisms is the organisation of tubules and vascular bundles inside the renal medulla, particularly within the inner stripe (papilla) of the outer medulla. This structure fuelled the concept of a simple versus complex medulla and, moreover, the description of several degrees of complex medulla. In species such as the rabbit with a simple medulla, tubular structures present in the medullary rays (thin descending, thin and thick ascending limbs of the loop of Henle and collecting ducts) run in the inter-bundle regions and receive a blood supply from a capillary plexus, physically keeping tubular and vascular structures separated. In the complex medulla in species such as *Psammomys* (a desert rodent), the thin descending limbs of short looped nephrons penetrate into the vascular bundles (Bankir & de Rouffignac, 1985). As a result, the structures formed by the blood vessels vascular bundles are composed of ascending and descending vasa recta and thin descending limbs of short looped nephrons; one ascending vasa recta contains one descending vasa recta and one short descending limb. The amount of penetration inside the vascular bundle by the short descending limbs varies; in the rat, the short descending limbs only come close to the bundle, whereas in mice such as *Psammomys* and *Merriones*, the short descending limbs penetrate the bundles. *Psammomys* have a greater level of complexity as the bundles fuse to form giant bundles and therefore the typical counter-current arrangement between ascending and descending vasa recta is replaced by complex, or giant bundles, in the papilla of the outer medulla (Bozinovic & Gallardo, 2006).

2.4   Measuring Kidney Function

Early detection of kidney disease is crucial for successful patient treatment and management; current diagnostics such as urine output and serum creatinine are insensitive, nonspecific, and late markers of disease (Siew et al., 2011; Zhang et al., 2013). Therefore, the discovery of specific, reliable diagnostic and prognostic biomarkers is essential for the early detection of kidney disease (Zhang et al., 2013). Direct measures of GFR are considered more accurate than biomarkers; however the use of techniques such as iohexol clearance, although practical, limitations often hamper its routine use in clinical practice (Paepe & Daminet, 2013; Quimby, 2015). A significant effort has been made to identify novel biomarkers of renal function, particularly for cats with CKD, however none have been identified that achieve a more

comprehensive picture than that offered by serum creatinine (Quimby, 2015). In clinical practice, plasma or serum creatinine, urea concentration, and urine specific gravity (USG) are routinely used markers of renal function (Finch, 2014).

2.4.1 Urine Specific Gravity (USG)

USG is the density ratio of urine compared to pure water at a constant temperature. The specific gravity of a solution is affected by the number of particles present in the same solution and their molecular mass. In normal urine, small molecular size substances (such as NaCl and urea) are mainly present, and substances with large and heavy molecules (such as glucose and albumin) are not usually found (Di Bella et al., 2014).

USG reflects the ability of the renal tubules to concentrate or dilute urine according to the body's needs to balance water intake and excretion. While renal mass influences maximal urine concentrating ability, non-renal factors including, intake of certain drugs (such as diuretics, corticosteroids), diet, or fluid intake can have a major influence on USG (Finch, 2014).

USG is considered an insensitive marker of renal function. Cats have a greater capacity to concentrate their urine than dogs or humans (Ross & Finco, 1981). Furthermore, USG can vary in cats with CKD, so it does not predict disease progression (Chakrabarti et al., 2012), and should not be considered a marker of glomerular filtration rate (GFR) (Finch, 2014).

Most cats with CKD have isothenuric urine (where USG is between 1.007–1.015) (DiBartola et al., 1987; Elliott & Barber, 1998). Isothenuria occurs when greater than 66% of nephrons have become non-functional (Chew et al., 2011). Some cats with spontaneous or experimentally induced CKD can retain their urine concentrating ability despite being azotaemic, particularly in the early stages of disease (Ross & Finco, 1981; DiBartola et al., 1987). As CKD progresses USG usually gradually declines (Elliott & Barber, 1998; Elliott et al., 2003).

2.4.2 Urea

Urea is one of the routine measures used in diagnosing CKD (Finch, 2014), however, even at the highest urine flow rate, approximately 40% of the filtered urea is reabsorbed making this a poorer marker of estimation of GFR when compared with creatinine (Backlund et al., 2011). Creatinine concentration is inversely related to GFR and is affected by fewer non-renal factors making it a preferred marker of GFR over urea (Sparkes et al., 2016).

2.4.3   Plasma or Serum creatinine

Creatinine (either plasma or serum) is the most widely used surrogate marker of renal function which is based on its relationship to GFR (Finch, 2014). Serum creatinine concentration is often used to estimate GFR and stage CKD (Cobrin et al., 2013; Finch, 2014), yet it is an insensitive marker of renal function which in turn is poorly correlated with GFR in early renal dysfunction (Haller et al., 2003).

Creatinine is formed from non-enzymatic dehydration of creatine phosphate in skeletal muscle at a stable rate directly correlated to muscle mass in healthy individuals (Perrone et al., 1992). Creatinine is also therefore a marker of muscle mass, and concentrations in plasma can consequently depend on muscle mass (Finch, 2014). As creatinine is not protein bound it is freely filtered by the glomerulus and, as it is not reabsorbed or secreted to any considerable extent by the renal tubules in cats, creatinine plasma concentration is inversely correlated with GFR (Finch, 2014). Circulating creatinine concentration is the most widely used marker of renal function in human and veterinary medicine (Cobrin et al., 2013). In patients with kidney disease creatinine generation may be reduced as the first step of creatinine biosynthesis takes place in the kidneys (Finch, 2014).

Creatinine concentrations may also vary depending on the type of sample collected (plasma versus serum) (Finch, 2014) and method of analysis. Some assays, such as Jaffe chemistry which detects non-creatinine chromogens, can report falsely increased creatinine concentrations (Von Hendy-Willson & Pressler, 2011; Finch, 2014). Additionally, when measured in different veterinary practices, creatinine concentrations can vary, especially when using bench-top analysers for the same assay (Braun et al., 2008). Braun et al. (2008) compared the measurements of two different concentrations of plasma creatinine by 99 veterinary practices in different regions of France; the results showed 20.2% of the measurements for the lower concentration sample and 5.8% for the higher concentration sample were outside the mean ± 15% range corresponding to the maximal allowable error according to the Clinical Laboratory Improvements Amendments. Variation in reference intervals between laboratories, can also lead to cats being classified differently and perhaps incorrectly depending on which laboratory undertakes the analysis (Ulleberg et al., 2011).

The relationship between GFR and creatinine is not particularly strong, explaining only 43% of the variability in GFR. Combining creatinine with urea (urea alone explains only 25% of the variability in GFR) improves the prediction of GFR but still only explains 48% of variability. Due to a non-linear relationship a significant decrease in GFR is required before corresponding increases in creatinine concentration are seen; a reduction in GFR from 3.5 to 1.5ml/min/kg resulted in a corresponding increase in plasma creatinine of only approximately 100 to 150µmol/l (Finch, 2014).

Non-renal factors, such as dehydration and a high protein diet, can also influence both urea and creatinine concentrations. Urea is passively absorbed in the renal tubules; so in situations of slow tubular flow rates such as dehydration or hypovolaemia (decreased blood volume), urea plasma concentrations can increase (Finch, 2014).

In most clinical settings, serum creatinine concentration measurement has replaced GFR measurement because of its simplicity and widespread availability; nevertheless, it is likely that serum creatinine does not increase above reference range until approximately 75% of nephrons are non-functional (Von Hendy-Willson & Pressler, 2011). The actual determination of GFR should therefore be considered superior due to the lack of sensitivity and specificity of creatinine for diagnosing kidney disease (Von Hendy-Willson & Pressler, 2011).

A comparison of GFR values with creatinine concentrations emphasises the need for more sensitive detection methods. Two old cats (in a study of 12 cats) were classified as normal based on creatinine values alone but had slightly reduced GFRs indicating they had early renal disease (Haller et al., 2003). Blood urea nitrogen (BUN) levels were also determined to be even less sensitive in detecting early renal dysfunction, as six of the 12 cats had a reduced GFR while BUN levels were in the normal range (Haller et al., 2003).

The limitations and lack of sensitivity of plasma creatinine, urea, and USG as markers of functional renal mass in identifying early kidney disease has led to demand for more accurate methods of renal function assessment. GFR is considered to provide the most accurate and sensitive evaluation of functional renal mass, and is useful in various clinical scenarios and in research settings to provide an accurate measurement of renal function. Although its application in these scenarios is yet to be fully evaluated, GFR may also be valuable for monitoring renal function following toxin exposure or recovery from acute kidney injury, (Finch, 2014).

2.5 Markers and techniques for measuring GFR

Several techniques for measuring GFR have been validated in both research and clinical settings. Current methods however can be complex, and may rely on multiple blood draws or 24-h urine collection which can also make them costly. As a result veterinary clinics still require simple and widely available methods for determining GFR (Von Hendy-Willson & Pressler, 2011).

A number of studies including healthy animals have published GFR results using techniques including; plasma inulin clearance (Fettman et al., 1985; Rogers et al., 1991; Brown et al., 1996; Haller et al., 2003), plasma exogenous creatinine clearance (Brown et al., 1996; Miyamoto, 2001b; van Hoek et al., 2007; Heiene et al., 2009; van Hoek et al., 2009; Reynolds et al., 2014), and plasma iohexol clearance (Miyamoto, 2001c; Goy-Thollot et al., 2006; Heiene et al., 2009; Paepe et al., 2015). 'Normal' GFR is poorly defined in cats, and a definitive reference range has not been agreed upon (Von Hendy-Willson & Pressler, 2011). This is most likely due to variations in protocols (including urine or blood sampling times, choice of markers and assays used, and pharmacokinetic models used for GFR calculation), as well as the unknown effect of individual factors which may influence GFR, for example dietary protein concentration, hydration status, patient signalment (veterinary medical history including the animal's age, sex and breed), circadian variation, and the use of sedation during GFR measurement (Von Hendy-Willson & Pressler, 2011).

One such technique used to determine GFR (and a "normal" range) is the measurement of clearance of a marker from blood plasma (plasma clearance). This technique refers to the amount of a 'marker' (an injected substance) removed from plasma over a given period of time and is expressed as the volume of plasma theoretically rendered 'clear' of the marker in mL/min. It is important that markers are; eliminated solely by the kidneys via glomerular filtration, neither reabsorbed or secreted by the tubules, and non-toxic, resulting in an accurate estimate of GFR as renal clearance is equal to plasma clearance (Von Hendy-Willson & Pressler, 2011).

The use of plasma clearance models has enabled simpler and faster techniques to be developed for measuring GFR rather than renal clearance and avoids the need for urine collection (Von Hendy-Willson & Pressler, 2011). Accurate plasma clearance methods rely on collection of blood samples at multiple time points to create a plasma concentration versus time curve from which the clearance is then calculated (Finch, 2014). To improve clinical feasibility of GFR measurement, attempts have focused on limiting sampling time-points for the determination of plasma clearance and comparing their validity with other more extensive methods. It has yet to be established how many samples are required to balance the requirements of the various pharmacokinetic models for measuring plasma clearance methods with potential clinical limitations and also depends on the GFR marker used (Von Hendy-Willson & Pressler, 2011).

2.5.1 Inulin

Inulin is a fructose polymer that comes very close to satisfying all the above important criteria for a plasma clearance technique, therefore renal clearance of inulin is considered by some researchers to be the gold standard for measuring GFR (Haller et al., 2003; Kerl & Cook, 2005). However, for inulin-based techniques, the drawbacks of assays used for measuring include being technically demanding and not commonly available. Because a complete and accurate 24-hour urine collection is required for inulin-based techniques, animals must therefore be housed in metabolic cages or repeatedly catheterised in order to remove all urine, which also has an associated risk of development of lower urinary tract infection (Miyamoto, 2001c; Haller et al., 2003). Catheter-associated bacterial urinary tract infection is a common complication of indwelling urinary catheters, particularly in open-ended systems. Uropathogens may be introduced into the urinary tract during diagnostic and/or therapeutic procedures and the risk of infection is further compounded if the patient has pre-existing urinary tract disease (Olin & Bartges, 2015).

While the use of inulin plasma clearance techniques is similar to that of renal clearance, it is also generally precluded due to drawbacks which include; the expense (Heiene et al., 2009), lack of inulin availability (Kerl & Cook, 2005), requirement for transport of frozen plasma samples (Heiene et al., 2009), low solubility that requires the collection of a large volume of blood (Katayama et al., 2012), and complex laboratory analyses (Kerl & Cook, 2005; Heiene et al., 2009; Katayama et al., 2012).

2.5.2 Iohexol

Different markers have been validated as alternatives to inulin, with iohexol the most commonly used (Goy-Thollot et al., 2006). Iohexol is an iodinated radiographic contrast agent that is eliminated solely through renal excretion following intravenous injection (Kerl & Cook, 2005). Studies using various methods for measuring iohexol in plasma have produced consistent results over a variety of GFRs, although some variation exists between the absolute values obtained (Von Hendy-Willson & Pressler, 2011).

Iohexol has a number of distinct advantages over other markers including stability, which allows samples to be sent to an external laboratory for analysis, more rapid elimination than other markers (such as injectable creatinine), a generally low toxicity, and low cost. Iohexol is also widely available because it is commonly used as a radiographic contrast agent, and its increasing popularity in human medicine for estimating GFR (Heiene et al., 2009).

2.6 Feline chronic kidney disease (CKD)

Feline CKD is a common disease affecting cats (Cobrin et al., 2013; Paepe & Daminet, 2013), particularly older/geriatric cats (Elliott & Barber, 1998; Chakrabarti et al., 2012). The overall prevalence of CKD is thought to range from 1.6-20% in cats (Lund et al., 1999; Polzin, 2010), rising to up to 32% in cats aged over 15 years (Polzin, 2010). CKD is defined as structural and/or functional impairment of one or both kidneys that has been present for approximately 3 months or more. Most cats will have a loss of function and structure with CKD; however, the degree of functional impairment is not always mirrored by loss of structure. CKD is an irreversible loss of renal function and/or structure that remains stable for some period of time but is ultimately progressive (Bartges, 2012). CKD occurs when compensatory mechanisms are no longer able to maintain the excretory, regulatory, and endocrine functions in animals with kidney disease (Nelson & Couto, 2014). For cats with CKD timely diagnosis and staging can facilitate the initiation of medical management improving the prognosis (Paepe & Daminet, 2013).

CKD is diagnosed based on the combined existence of: renal azotaemia (increased blood urea due to decreased GFR), poorly concentrated urine (USG ≤1.035), and compatible historical and/or physical examination findings (Bartges, 2012; Nelson & Couto, 2014). Based on complete blood and urine examination the diagnosis of advanced feline CKD and accompanying complications is typically straightforward, although the limitations of routine blood and urine tests make detecting and diagnosing early or non-azotaemic CKD much more

challenging than later stages of CKD (Paepe & Daminet, 2013) since azotaemia does not develop until 75% or more of nephrons have become non-functional (Chew et al., 2011).

Current clinical practice guidelines for diagnosis, prognosis and treatment of CKD are largely based on the stage of the disease (Polzin, 2013). Staging of CKD follows guidelines developed by the International Renal Interest Society (IRIS). IRIS CKD staging is a 4 tier system (see Figure 2.5 below) based on serum creatinine concentration, the degree of proteinuria (urine containing an abnormal amount of protein) as measured by the urine protein: creatinine ratio (UPC) and blood pressure (Polzin, 2013).
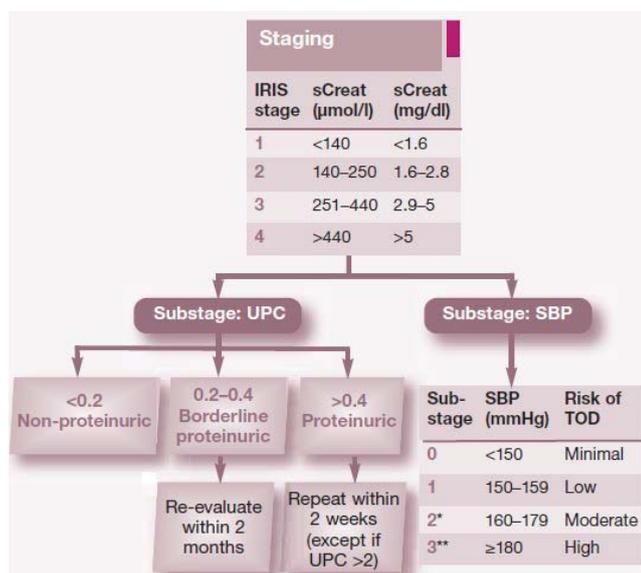
Figure 2.5 - Overview of IRIS staging for feline patients with CKD - The serum creatinine concentration (sCreat) determines the IRIS stage. Further sub-staging is based on the degree of proteinuria, assessed by the urinary protein: creatinine ratio (UPC) and systolic blood pressure (SBP). The SBP sub-staging system reflects the risk of target organ damage (TOD) (Paepe & Daminet, 2013) (Reproduced by permission of author and right's holder).

*Persistence of elevation should be judged on multiple blood pressure measurements over a period of 2 months, **Persistence of elevation should be judged on multiple blood pressure measurements over a period of 1–2 weeks

IRIS staging has been strongly associated with survival time. Depending on the stage and progression of disease, the reported median survival times (MSTs) with CKD varies widely; with those animals with a lower IRIS stage living longer (1151 days, Stage 2 at time of diagnosis) than those with more advanced stages (778 days for Stage 3, and 103 days for Stage 4) (Boyd et al., 2008).

While proteinuria contributes to a decreased survival time in cats, unlike dogs, hypertension has not been thought to be associated with decreased survival time (Syme et al., 2006; Jepson et al., 2007; King et al., 2007). More recently this link has been reconsidered; it is now thought that while there is a generally presumed link between hypertension and kidney disease, it remains unclear what role feline systemic hypertension plays in the cause and progression of CKD (Syme, 2011).

The guidelines for diagnosing suspected feline CKD are also designed to; identify potential underlying aetiologies of CKD that may require specific therapy; identify complications arising from CKD and to identify simultaneous disease that may affect management, e.g. hyperthyroidism. The recommended minimum diagnostics include; a full history and physical examination; routine urinalysis including USG, 'dipstick' analysis, urine sediment analysis; urine protein: creatinine ratio (UPC), and where indicated urine culture; routine serum biochemistry, including proteins, urea, creatinine, electrolytes (Na^+, K^+, Ca^{2+}, Cl^-, PO_4), and other analytes as relevant (e.g., thyroxine in older cats); routine haematology; systolic blood pressure (SBP) and diagnostic imaging (renal ultrasonography is generally more valuable than radiography) (Sparkes et al., 2016).

2.7 Composition of foods

2.7.1 The role of diet

While the primary role of a diet is to provide enough nutrients to meet metabolic requirements, it is thought that beyond simply meeting nutritional needs (Bontempo, 2005), diet may have detrimental or beneficial roles in some diseases (Bontempo, 2005). In diabetes for example, the change to modern high carbohydrate diets from historical low carbohydrate diets (as discussed in section 2.7.2), and a shift from an outdoor environment to an indoor setting and the accompanying decreased physical activity because cats no longer need to hunt to obtain food (Verbrugghe et al., 2012), is believed to have a causal effect on diabetes due to

an association with insulin resistance (Verbrugghe et al., 2012; Villaverde & Fascetti, 2014). However, diet is also thought to have a beneficial management role in glycaemic response (Villaverde & Fascetti, 2014) and prevention of the disease by preventing insulin resistance, through the alteration in diet composition (Verbrugghe et al., 2012). Concepts in nutrition have expanded to include an emphasis on the use of foods to promote a state of well-being, better health, and to help to reduce the risk of diseases. Maintaining health and seeking optimal nutritional products are seen by pet owners as important components of responsible pet ownership. It is therefore important to review the nutritional basis on which commercially prepared foods are formulated (Bontempo, 2005).

Progress in establishing the nutrient requirements of cats has resulted in a large number of excellent commercially prepared pet foods supporting the growth, reproduction and maintenance of healthy companion animals (Bontempo, 2005). Further development has resulted in foods that are designed for specific stages of life, physiological, and disease states. Some of the more recent niche foods include; foods that target the health of specific body systems, or those patterned after popular human diets which include organic ingredients, or various types of raw or grain-free diets (Case et al., 2011; Buff et al., 2014). These trends represent a response to the owner's desire to supply their cats with the best nutrition possible during all stages of life (Case et al., 2011).

2.7.2   Diet composition

Commercial diets are formulated with a mixture of animal- and plant-derived nutrients (Zoran, 2002). In contrast, the natural diet of wild or feral cats includes a wide variety of invertebrates, reptiles, birds and mammals, including prey up to their own body mass or size (Fitzgerald & Karl, 1979; Paltridge et al., 1997; Dierenfeld et al., 2002). Although cats have adjusted to most manufactured diets, there are limitations (as discussed below) associated with substituting nutrients of animal-origin with that of plant-origin (Zoran, 2002).

A pet food can be a single ingredient treat or a highly complex, 60-plus ingredient product (Thompson, 2008). Many modern commercial diets are typically formulated with high carbohydrate containing ingredients (Zoran, 2002; Verbrugghe et al., 2012), more so in dry foods which require carbohydrate for the expansion and cooking process (Zoran, 2002). In contrast, typical prey diets, as would be consumed in the wild, are generally very low in carbohydrate, and high in crude protein and crude fat (Verbrugghe et al., 2012); for example an adult domestic mouse is 55.8% crude protein, 23.6% crude fat, 8.8% nitrogen free extract

(carbohydrate) and 11.8% ash on dry matter (DM) basis (Dierenfeld et al., 2002). Comparing the nutrient composition of some of these wild foods to The Association of American Feed Control Officials (AAFCO) standards (as discussed below in Section 2.8.1) (AAFCO, 2015) an adult domestic mouse or adult rat has sufficient Vitamins A and E, Ca, P, Mg, Cu, Fe & Mn but is deficient in Zn, while a rabbit has sufficient Ca, P, Mg, Na, Fe & Zn, but is deficient in Vitamin A, Cu, and Mn (Dierenfeld et al., 2002). This demonstrates that in the wild, cats eating what is available to them, may not meet nutrient requirements in a single-source meal unlike modern commercial diets. Instead overall they could meet their requirements by eating a number of meals of different wild foods.

2.8   Pet Food Regulations

2.8.1   Worldwide

AAFCO is one of the most significant agencies in the regulation of commercial pet foods. Formed in 1909, AAFCO sets standards for substantiation claims and provides an advisory committee for state legislation in the USA. AAFCO is not an official regulatory body but does operate within the guidelines of federal and state legislation including laws administered by the Food and Drug Administration (FDA) and the US Department of Agriculture (USDA) (Case et al., 2011). Each year AAFCO produces an Official Publication, which is the basis for pet food regulations in the USA and in some countries internationally. This publication specifies labelling procedures, ingredient definitions, and nomenclature for all animal feeds and pet foods. Outside the USA compliance with these standards is voluntary (Chandler & Takashima, 2014).

State feed agencies, the FDA, and the USDA provide guidelines, regulations, and oversight to ensure that pet foods are safe and nutritious. The Federal Food, Drug, and Cosmetic Act, administered by the FDA, require that pet foods are not adulterated or misbranded. Adulteration includes the presence of chemical, physical, or microbiological contaminants in the product, or the use of an ingredient that is not sanctioned for use in pet foods. Pet food ingredients are either accepted food additives (for human consumption), or included in the AAFCO Feed Ingredient Definition list (published annually) which is maintained jointly by AAFCO and the FDA. The inclusion in a pet food of any ingredient or additive that is not approved, or been accepted as safe via FDA regulatory discretion, is considered to be prohibited and constitutes adulteration (Case et al., 2011).

The FDA maintains a monitoring program that tests pet foods and pet food ingredients for the presence of pesticides, mycotoxins, and heavy metals through its Feed Contaminants Program. However, following several pet food recalls in recent years (the most extensive of which occurred in 2007 with more than 150 affected brands in the USA), both owners and professionals are now questioning the effectiveness of the quality and safety assurance programs that are currently in place (Case et al., 2011). In addition, these monitoring programmes do not cover all heavy metals and regulatory action for elements in feed and feed ingredients is decided on a case-by-case basis (Food and Drug Administration, 2010).

In Europe the European Pet Food Industry Federation (FEDIAF), established in 1970, represents the pet food industry in 26 European countries via 18 national or regional pet food industry associations. FEDIAF promotes the views and interests of approximately 650 European pet food producing companies and, working closely with the European Union authorities, aims to ensure a legislative framework for the production of safe, nutritious and palatable pet food which takes into account the specificity of the industry and its products. In 2001 FEDIAF adopted an internal set of standards which companies adhere to in order to prevent, rather than react to, potential difficulties in the production process and to meet their commitment to nutrition, safety and quality. Unlike in the USA, the European Union has legislative standards for heavy metals in both human and animal food stuffs, including maximum levels of cadmium (Cd) in pet food as detailed in Section 2.12 below (European Pet Food Industry Federation).

2.9   Cadmium in the environment

Cadmium is an impurity that is naturally present in phosphate rock, and consequently, in all phosphate fertilisers (Cadmium Working Group, 2008; Stafford et al., 2014). Phosphate fertiliser was, and continues to be, a critical component of increasing productivity in New Zealand agriculture (Cavanagh et al., 2013). Other environmental sources of cadmium include naturally heavy metal rich soils where metal ores come to the surface and decay due to weathering, ore mining, industrial and vehicle emissions (Sigel et al., 2013), vehicle tyres, and cigarette smoke (Duran et al., 2010). As a consequence of environmental sources and in particular the application of mineral fertilisers (He et al., 2005), cadmium has been detected in drinking water and processed meats (Duran et al., 2010). When grown on a cadmium-polluted soil, some crops, such as rice, can accumulate substantial amounts of cadmium (>1000µg/kg) (World Health Organization Regional Office for Europe, 2000). Animals grazing on fertilised lands have the potential for high cadmium levels in their tissues (Paßlack et al., 2014).

The highest cadmium levels have been found in mammalian organ meats, particularly kidney and liver, and in certain species of mussels, scallops and oysters (World Health Organization Regional Office for Europe, 2000). In New Zealand, industrial exposure to cadmium is rare; the main sources of (human) exposure are through tobacco products and food (Cadmium Working Group, 2008). However, in pet foods the number of constituents that can be included in a single commercial diet, increase the possibility of potential contamination by agricultural and industrial pollutants from the cultivation and/or production of the plant and animal ingredients, as well during final processing of the finished product (Bontempo, 2005).

With repeated application, cadmium tends to accumulate in soil, although the accumulation rates vary between regions of New Zealand due to a number of variables including; differences in land use history, fertiliser cadmium content, total fertiliser use, soil types, and climate (Cadmium Working Group, 2008). In New Zealand, cadmium accumulation in agricultural land was first identified as a long-term management issue in the early 1990s (Stafford et al., 2014), when it was discovered that ≈20% of sheep and cattle liver and kidneys exceeded the Maximum Permissible Concentration of 1mg Cd/kg fresh weight (FW) as set by the Ministry of Health at the time (Loganathan et al., 2003). This resulted in an offal discard policy being implemented and was followed by the introduction of voluntary limits on the level of cadmium in phosphate fertiliser (Cavanagh et al., 2013). Prior to the 1990s phosphate fertilisers had high cadmium levels ranging from 200-490mg Cd/kg phosphorus (P) (Cadmium Working Group, 2008), following the introduction of voluntary limits, the lower limit of cadmium content in fertiliser was reduced down to 340mg Cd/kg P in July 1995 and then to 280mg Cd/kg P by Jan 1997. An independent audit carried out between January 2001 - June 2005 showed that the weighted average of cadmium content of phosphate fertilisers was between 149mg cadmium/kg P and 193mg cadmium/kg P (Cadmium Working Group, 2008).

In 2003 the *Guidelines for the safe application of biosolids to land in New Zealand* were published (New Zealand Water & Wastes Association, 2003); these guidelines included a soil limit of 1mg Cd/kg dry weight of soil for the application of biosolids to land. This was aimed at minimising cadmium concentrations in animal and crop products and to avoid barriers to international trade. It should be noted that these guidelines have no legal status (Cadmium Working Group, 2008; Cavanagh et al., 2013); therefore, it is up to local authorities to translate any published guidelines into a legally enforceable standard (Cadmium Working Group, 2008).

A 2007 report on the cadmium content of New Zealand soils (Taylor, 2007) found that pastoral land-uses had the highest individual soil cadmium levels (2.70µg/g). Dairying had the highest

average soil cadmium concentrations (0.73µg/g) which was double the national average (0.35µg/g) and had the widest range of cadmium values measured. Average values for beef farming and all dry stock were slightly above the national average (0.42µg/g and 0.40µg/g respectively), while sheep farming was slightly below (0.33µg/g). Both sheep and beef farming led to more accumulation of cadmium in soils than dairy when both used the same fertiliser regime; however accumulation was higher under dairy when under their respective optimal fertiliser schemes (Taylor, 2007).

2.10 Cadmium and Heavy Metal Toxicity

2.10.1 Heavy Metals

Metals such as iron (Fe), copper (Cu) and zinc (Zn) are important for normal health (Johri et al., 2010). They participate in the regulation of a number of physiological functions including; nucleic acid and protein synthesis, enzymatic reactions, membrane stabilization, immune system function, and antioxidant defence. These metals are effective at very low concentrations, and their concentration in the body must be tightly regulated, as either deficiency or excess can cause severe illness and death (Barbier et al., 2005).

In contrast, heavy metals including mercury (Hg), lead (Pb), and cadmium (Cd) are non-essential and potentially toxic, especially if they accumulate in an animal (Johri et al., 2010) These elements can be toxic at very low doses and have very long biological half-lives; accordingly, exposure to heavy metals is potentially harmful (Barbier et al., 2005). Mercury is primarily associated with neurological effects resulting from lesions in the cerebral cortex and cerebellum (Charbonneau et al., 1976). Lead is associated primarily with toxic effects in hepatocytes and renal tubular epithelial cells by complexes formed with sulfhydryl groups in critical sulfhydryl-dependent proteins resulting in enzyme inhibition and eventually cell death (Knight & Kumar, 2003). In contrast cadmium is associated with a range of pathologies, as outlined in Section 2.10.2.

2.10.2 Cadmium-Associated Pathology

Cadmium does not have any known role in biological systems (Andreani et al., 2010), and has a low rate of excretion and therefore a long biological half-life (10–25 years) (Dudley et al., 1985). Cadmium accumulates in the liver, kidneys and bones following chronic exposure, with about 50% accumulating in the kidneys (Johri et al., 2010). The lack of efficient export systems

(Martelli et al., 2006) and chronic cadmium toxicity may have severe effects in the kidney (nephropathy and proteinuria) and in bones (osteoporosis). A variety of cancers including leukaemia and lymphoma, and cancers of the liver, lungs, and kidney, have also been associated with cadmium exposure (Huff et al., 2007). There is also emerging evidence to suggest that cadmium is genotoxic (Anetor, 2012), and is associated with poor healing and premature ageing (Lafuente, 2013).

Bone decalcification and increased urinary excretion of calcium are also associated with chronic cadmium exposure. Adverse effects on bone and calcium metabolism may be a direct result of cadmium consumption, or secondary to the renal damage and subsequent interference with calcium metabolism and kinetics (Ministry for the Environment, 2011; Sigel et al., 2013). This may lead to osteomalacia (softening of the bones) or osteoporosis (increasingly brittle bones) (Ministry for the Environment, 2011; Agency for Toxic Substances and Disease Registry, 2012). Skeletal manifestations have also been partially linked to cadmium nephrotoxicity (Johri et al., 2010).

The kidneys are particularly susceptible to cadmium toxicity (Johri et al., 2010), where cadmium accumulates over time and may cause renal dysfunction due to damage to the proximal tubular cells. Increased levels of low-molecular-weight proteins such as β2-microglobulin in the urine (tubular proteinuria) are the first sign of this dysfunction (Bernard, 2004). Even after cadmium exposure has stopped, cadmium-induced proteinuria is not readily reversible and progresses, likely due to the redistribution of cadmium accumulated in the liver to the kidneys (Ministry for the Environment, 2011).

While cadmium accumulation has been studied in depth across human populations, there is a lack of information regarding accumulation in cats. One study analysed the concentrations of four metals (Zn, Cu, Fe, and Cd) and isolated metallothioneins (MTs) in the liver and kidney of cats that had died from different pathologies (Andreani et al., 2010). Higher cadmium concentrations were found in kidneys compared to livers, and the levels of cadmium were positively correlated with age in both tissues. This suggests chronic exposure of the cats to cadmium, and three of the 23 cats examined in the study appeared to have been exposed for longer or to higher concentrations which the authors attributed to dietary factors (Andreani et al., 2010).

2.10.3 Concentration/dose-response relationship of cadmium toxicity

Tubular dysfunction usually develops in humans only after the cadmium concentration in the renal cortex reaches a critical level (Järup et al., 1998; Ministry for the Environment, 2011). For example, the urinary concentration of cadmium associated with the onset of renal injury is approximately 2–10μg/g creatinine, which corresponds to a renal cortical cadmium concentration of about 150–200μg/g tissue (Sigel et al., 2013). While the critical tissue concentration of cadmium at which renal injury occurs is subject to inter-individual variation, it has been suggested that a value of 200μg Cd/g wet weight in the renal cortex corresponds to a 10% incidence of proteinuria (Ministry for the Environment, 2011) and is estimated to correspond to an approximate urinary cadmium concentration of about 10μg/g creatinine (Järup et al., 1998). There appears to be three urinary cadmium thresholds for the development of cadmium-induced renal effects; biochemical changes predominantly occur above 2μg/g creatinine; above approximately 4μg/g creatinine glomerular barrier function is compromised and cytotoxic effects appear in the proximal tubules; and the onset of proteinuria occurs at around 10μg/g creatinine (Ministry for the Environment, 2011). In their most recent evaluation of cadmium, The Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organization stated that the critical health outcome with regards to the toxicity of cadmium is renal tubular dysfunction, and that an excess prevalence would not be expected to occur if urinary cadmium concentrations remain below 2.5μg/g creatinine (Ministry for the Environment, 2011).

In Europe, it is estimated that cadmium levels in the renal cortex of the general population aged 40–60 years are 15–40μg/g (World Health Organization Regional Office for Europe, 2000), and values of 72-84μg/g have been reported in older people in non-polluted areas of Japan (Uetani et al., 2006; Sigel et al., 2013). The urinary concentration of cadmium in otherwise healthy individuals is <1μg/g of creatinine and is proportional to the cadmium content of the kidney, yet excretion is not significant enough to rid the body of cadmium as the total daily excretion through all pathways (urine, intestines, sweat) represents <0.01% of the total body cadmium (Sigel et al., 2013). Toxicokinetic modelling for cadmium has typically been used to estimate tolerable intakes (for humans) (Ministry for the Environment, 2011).

2.10.4 Cadmium effects on the kidney

The renal accumulation of cadmium takes place mainly in the proximal tubules where the metal gives rise to a dose-dependent toxicity over a threshold concentration (see Section 2.10.3 above). While the proximal tubule has long been recognised as a major target in cadmium-induced nephropathy, other segments of the nephron can also be affected but usually at more advanced stages of toxicity than the proximal tubules (Nordberg, 2009).

Renal glomeruli are also exposed to circulating metals during plasma filtration, and may also be targets of cadmium toxicity (Templeton & Chaitu, 1990; Dorian et al., 1992; Uriu et al., 1998). The mesangial cell (specialised smooth muscle (SM) cells that regulate blood flow through the capillaries of the kidney) has an abundance of α-SM actin (α-SMA) as well as β actin; together they form a structured cytoskeleton with prominent stress fibres and a cortical actin band in cell culture. As a contractile cell anchored to the mesangial angles of the glomerular capillary network, it is thought to play a role in regulating capillary cross-sectional area and therefore glomerular filtration rate. In studies on rat, mouse, and human mesangial cells, cadmium was shown to affect several death and survival pathways and its effects on the actin cytoskeleton were the likely mechanisms modulating these processes. In the context of cadmium nephrotoxicity it was noted that cadmium effectively disrupted F-actin filaments in cultured mesangial cells while other divalent metals such as manganese, cobalt, nickel, copper, or zinc had no effect (Templeton & Liu, 2013).

2.10.5 Molecular mechanisms of cadmium toxicity

At the molecular level, cadmium can bind to, and interfere with, a large number of transporters (for example, the ABC family of detoxification transporter pumps, voltage-dependent Ca^{2+} channels, or Ca^{2+} carriers), signalling molecules (such as growth factor or hormone receptors) (Sigel et al., 2013), enzymes (for example $Na^+ K^+$-ATPase (Kerkhove et al., 2010)), transcription factors (including zinc containing transcription factors) and metalloproteins (e.g. Zn-, Ca-, Fe-, Mn-, and Cu-binding proteins (Sigel et al., 2013). Theoretically using these transport systems allows cadmium to be absorbed and distributed, disrupting homeostasis (Moulis, 2010).

In general, metal binding sites within biological molecules have low affinity and lack specificity, although metalloproteins and transporters for essential metals may have greater selectivity or indeed may be specific (Sigel et al., 2013). As such, the effects of cadmium are likely to be apparent only at high concentrations. Thus *in vitro* experiments that utilise high concentrations

to define mechanisms may have limited, if any, relevance for the lower tissue concentrations that are achieved *in vivo* (Sigel et al., 2013). Metallothionein (MT) is an abundant protein in the animal kingdom. Its physiological functions are not fully elucidated (Klaassen et al., 1999); however, given its high affinity for divalent heavy metal ions, MT plays an important role in regulating metal homeostasis (Vašák, 2005). Several studies have suggested that MT plays an important role in cadmium disposition and detoxification (Klaassen et al., 1999).

Cadmium is transported to the liver bound to high-molecular weight proteins within plasma (Nordberg, 2004). Once in the liver it stimulates production of MT forming a Cd-MT complex which has a further high affinity for more cadmium. Cd-MT is then released into the blood and then redistributed through circulation to the kidneys (Dudley et al., 1985), where it is filtered at the glomerulus and subsequently reabsorbed by renal tubular cells (Nordberg, 2004). Once in the proximal tubular cells it can cause nephrotoxicity (Dudley et al., 1985) either due to damage to cells by Cd-MT as it is being reabsorbed, or the Cd-MT may be degraded by lysosomes releasing unbound cadmium, some of which is excreted in urine (Nordberg, 2004). Only a minor proportion of the total body burden of cadmium is excreted and progressive accumulation occurs with age (Johri et al., 2010). Cd-MT in the kidney has been shown to be the most sensitive marker for cadmium exposure when compared to antioxidant status and lipid peroxidation (Hispard et al., 2008).

Oxidation reduction reactions (redox) are chemical reactions in which atoms have their oxidation state changed; in general, redox reactions involve the transfer of electrons between a molecule, atom, or ion (Ercal et al., 2001; Rani et al., 2014). Although cadmium does not undergo redox cycling, it does increase cellular levels of reactive oxygen species (ROS) that trigger apoptosis (Oh & Lim, 2006). Cadmium also depletes the cell's major antioxidants, particularly thiol-containing antioxidants and enzymes, resulting in a condition known as oxidative stress (Ercal et al., 2001; Rani et al., 2014). Oxidative stress may be partially responsible for the toxic effects of heavy metals as it induces various dysfunctions due to lesions caused by ROS to lipids, proteins and DNA (Ercal et al., 2001). While the depletion of antioxidant defences might seem to be a plausible mechanism of cadmium mediated damage, cadmium also facilitates adaptive increases in glutathione and MT that are protective against oxidative stress (Liu et al., 2009).

Since metal homeostasis is deregulated and ROS are produced during most disease processes, and both metals and ROS are strong inducers of MT expression, it is not surprising that MT levels vary in response to several diseases. As a result these proteins have been proposed as

useful biomarkers in the medical field (and more recently in the veterinary field), to measure environmental metals exposure, and especially for types of cancer such as human breast and bladder and canine apocrine cancers (Bay et al., 2006; Carpenè et al., 2007; Hinkel et al., 2008; Martano et al., 2012). As such MT has been used in a number of studies as a biomarker of both cadmium exposure and of cadmium induced proximal tubular injury (Prozialeck & Edwards, 2010).

2.11 Cadmium and Food Safety

A variety of toxicological endpoints (e.g. reproductive toxicity, neurotoxicity and carcinogenicity) have been observed and investigated in experimental animals and human populations (International Programme on Chemical Safety, 1992; Järup et al., 1998; Hopkins & Hosford, 2009; Agency for Toxic Substances and Disease Registry, 2012). Dietary cadmium intake by humans through food or drink in concentrations in excess of ≈15mg/kg results in acute gastrointestinal symptoms, including vomiting, diarrhoea, and abdominal cramps (Hopkins & Hosford, 2009). Estimated intakes for New Zealand are; for a child (aged 1–3 years, 13 kg) 12.5μg/kg body weight (BW)/month, and for an adult 7.9μg/kg BW/month (Ministry for the Environment, 2011). However, the critical health effect in humans is considered to be the adverse effects on kidneys as a result of long-term low-level exposure to cadmium (Järup et al., 1998; World Health Organization, 2011).

In humans despite cadmium absorption after dietary exposure being relatively low over time (3–5%), this low exposure may cause accumulation, particularly in the kidneys. Both the kidneys and liver act as cadmium stores (collectively storing 50–85% of the body burden and with 30–60% stored in the kidneys alone); however liver stores are gradually released to the kidneys (Ministry for the Environment, 2011; World Health Organization, 2011). Because it is possible to identify a level of exposure, at or below, which it does not induce an adverse effect, cadmium is considered to be a threshold contaminant and toxicological intake values generally propose a lifetime daily exposure level where there is no substantial risk to human health (Ministry for the Environment, 2011).

JECFA recommended a provisional tolerable weekly intake for humans (PTWI) of 7μg/kg BW/week. This value and the derived total daily intake (TDI), has been the value most widely used by various international agencies (Ministry for the Environment, 2011). However, it is recognised that the general population may not sufficiently protected by this value (Järup et

al., 1998; Satarug & Moore, 2004; Satarug et al., 2010) resulting in some agencies adopting different values. The Netherlands applied an additional safety factor of 2 to the JECFA value, while the Agency for Toxic Substances and Disease Registry (ATSDR) in the USA derived a TDI of 0.1µg/kg BW/day based on toxicokinetic modelling (Ministry for the Environment, 2011). The most recent estimate is that of the European Food Safety Authority (EFSA), who also using toxicokinetic modelling, derived a PTWI of 2.5µg/kg BW (Panel on Contaminants in the Food Chain, 2009). As a result of the EFSA decision JECFA reviewed and withdrew the previous PTWI in June 2010 and replaced it with a provisional tolerable monthly intake (PTMI) of 25µg/kg BW (0.8µg/kg BW/day) (Ministry for the Environment, 2011).

2.12 Cadmium in the food chain

Heavy metals, such as cadmium, Zn, Cu, and Pb, are a group of potentially toxic compounds that have been found in animal feedstuffs, particularly roughage. The degree to which these metals are available for plant uptake and further transfer into the food chain depends on the degree of pollution and soil characteristics; these factors result in varying regional differences in contamination levels of plants used for feed production (van der Fels-Klerx et al., 2011).

Carry-over of potentially toxic compounds, such as cadmium, from feed into consumable animal products, such as liver, kidney, and meat, can significantly contribute to the intake of these compounds. The cadmium uptake rate by plants from soil is higher than for any other toxic metal including mercury or lead (Satarug et al., 2006). Therefore, maximum limits have been legislated by the European Union for a number of potentially toxic compounds, including heavy metals, in animal feedstuffs (2002/32/EC) and animal derived food products (2001/466/EC) (van der Fels-Klerx et al., 2011). The most recent update in 2005 (2005/87/EC) legislated a maximum permitted content of 2mg Cd/kg in complete pet foods.

Within an animal, cadmium may accumulate in the organs and/or be present in milk, meat, and eggs. In livestock, cadmium is known to accumulate essentially irreversibly in the liver and kidneys. The extent of deposition and elimination in the animal depends on the half-life of the compound and the period considered (van der Fels-Klerx et al., 2011).

An Australian study examined 4973 kidneys from sheep stratified by age and location within an agricultural region, 483 aged sheep from a pastoral region, and 354 adult cattle from the Kimberley Region of Western Australia (statistical regions used for stratification of farms and pastoral holdings selected for sampling), and analysed cadmium content during the period

August 1989 to April 1991 (Morcombe et al., 1994). In the survey of the agricultural region, 72% of adult ewe (older than 4 years of age), 22% of 4-tooth ewe (28-34 months of age), 2% of hogget (16-22 months of age), but none of the Ewe lamb (4-10 months of age) kidneys exceeded the Australian maximum permissible concentration of 2.5mg/kg. In contrast, no kidney from adult ewes grazed in the pastoral region had cadmium concentrations greater than 2.5mg/kg. This data is consistent with previous estimates that 38% of sheep kidneys had cadmium concentrations exceeding 2.5mg/kg (Morcombe et al., 1994).

In New Zealand, kidneys from older cattle and sheep, aged 30–36 months old as identified by dentition (six or more permanent incisor teeth), are excluded from human consumption due to the likelihood of elevated cadmium levels (Cavanagh et al., 2013; Ministry for Primary Industries, 2013; Stafford et al., 2014). However these kidneys can be diverted for use in pet foods or meat and bone meal production on the basis that kidneys would be subject to significant dilution in the final products (Ministry for Primary Industries, 2013).

Heavy metal contaminants including cadmium and mercury have been found in meat and bone meal, and while the reason is for this contamination is not clear, one possibility is the inclusion of contaminated offal such as liver and kidney. Meat and bone meal is the most common protein supplement in dry dog foods in Australia with over 24,000 tonnes being used annually (Costa, 1997).

2.12.1 Cadmium in cat food

The FDA has no published guidelines, action levels, or tolerances for maximum cadmium content in cat food and instead refers to the information/recommendations published by the National Research Council of the National Academies (NRC) Committee on Minerals and Toxic Substances in Diets and Water for Animals (MTSA Committee). The FDA refers to these recommendations published in the *Mineral Tolerance of Animals (Second Edition)*, 2005, on the basis that the values recommended are based on results from studies in a wide variety of animals, including cats (Food and Drug Administration, 2011). Whilst there are numerous references in the *Mineral Tolerance of Animals* to the effects of cadmium exposure and concentrations in fluids and tissues of animals, there are no references directly pertaining to the cat. Furthermore no actual recommended cadmium limits are given for pet foods, although there is a 1mg/kg upper limit for cadmium in complete feeds for animals in order to prevent high levels in food products (National Research Council (U.S.), 2005). This presumably refers to agricultural species which will enter the food chain, and is referenced to a WHO

publication (International Programme on Chemical Safety, 1992). In this WHO publication (International Programme on Chemical Safety, 1992) there is only one study referenced looking at cadmium exposure in cats where it was reported that feeding 100mg Cd/day for one month resulted in varying degrees of desquamation in the proximal tubular epithelium (Prodan (1932) as cited in International Programme on Chemical Safety, 1992).

A recent study examined levels of cadmium in three wet cat foods and detected levels of between 0.94 - 2.14µg/g on a dry weight basis. These diets were predominantly flavoured as either chicken (origin Australia 1.15µg/g ± 0.17), or fish (origin USA 0.94µg/g ± 0.13, or South Africa 2.14µg/g ± 0.23) (Duran et al., 2010). A subsequent study suggested that cats eating one small 175g can of wet food per day would consume 13µg Cd/day (Atkins et al., 2011), which is >3x the reference dosage (RfD) values set by the US Environmental Protection Agency (EPA) and the permissible tolerable daily intake (PTDI) values set by the World Health Organization (WHO) for humans. Later analysis by the FDA disputed these results, concluding that while the authors used inductively coupled plasma-mass spectrometry to current scientific methodological standards to obtain the concentrations reported in the manuscripts, there were critical mistakes made in the approach taken to interpret the analytical results that confounded the scientific conclusions (Food and Drug Administration, 2011). In the recalculations published by the FDA, the cadmium levels in the 14 wet cat foods were considerably lower and ranged between 0.31 - 0.64µg/g/DM, the key problem with these values is that potentially they also involve measurement error. The FDA were forced to rely on estimations of a typical range of caloric density in the specified type of diet, because the authors of the original study did not publish nutrient profiles or a metabolisable energy (ME) value of the diets analysed. This means that neither set of calculations of cadmium intake could be considered accurate.

2.13 Cadmium and CKD

Although potential mechanisms have been outlined earlier, little evidence is available on how cats may be exposed to cadmium (Finch et al., 2012), the cadmium levels in the organs of cats (Paßlack et al., 2014), and how this relates to potential pathologies, particularly of the kidneys and/or liver (Andreani et al., 2010; Finch et al., 2012; Paßlack et al., 2014). It is important to evaluate if the accumulation of cadmium is dependent on kidney function and in particular, disorders in the renal excretory mechanisms and whether they may either increase or decrease cadmium deposition in the kidneys of cats (Paßlack et al., 2014).

Paßlack et al. (2014), measured the concentrations of cadmium as well as a number of other metals in the liver, renal cortex and renal medulla, and evaluated the impact of age, sex or the occurrence of CKD in cats. The study examined post-mortem concentrations in the tissues of 47 cats (22 males; 25 females; aged between 2 months and 18 years). Comparable cadmium concentrations were found in the liver and renal cortex of the cats, while lower amounts were detected in the renal medulla. Higher cadmium concentrations (liver; renal cortex) were also found in cats with CKD compared to healthy animals in the study. In addition, female cats had higher cadmium concentrations in the cortex of the kidneys compared to male cats, which is in agreement with data from humans (Kjellstrom, 1979). Cadmium concentrations in the liver and renal cortex were also age dependent, with lower concentrations in cats aged 0-7 years compared to those aged 12-18 years, which is also in agreement with another feline study (Andreani et al., 2010).

The study design of Paßlack et al. (2014), did not include the measurement of MT; and there appears to be only one study which has isolated and measured MT in tissues of cats (Andreani et al., 2010). The authors concluded that more studies are required to evaluate the potential of MT as a marker for the exposure of cats to specific elements including cadmium (Paßlack et al., 2014). It has been suggested that binding of cadmium to MT is a protective response to the toxic effects; however, once this pathway is exhausted, free cadmium may result in toxic injury (Johri et al., 2010). It is therefore possible that cadmium exposure may play a role in the development of feline CKD (Finch et al., 2012).

In cats with CKD, hypertension is frequently documented with the prevalence reported to be between 19-61% (Kobayashi et al., 1990; Syme et al., 2002; Brown et al., 2007). Furthermore, the prevalence of idiopathic feline hypertension is reported to be approximately 20% (Brown et al., 2007). Conflicting results in experimental animal and human studies have led to the suggestion that the hypertensive effects of cadmium are evident only at low-doses (Nakagawa & Nishijo, 1996), rather than high-dose cumulative exposure (Finch et al., 2012). A later systematic review of studies in humans found a positive relationship between blood cadmium concentrations and blood pressure, concluding that the relationship between urinary cadmium concentration and blood pressure remains unclear (Gallagher & Meliker, 2010). Multiple causalities of cadmium-induced hypertension have been speculated including; cadmium nephrotoxicity (Eum et al., 2008), renal tubular injury resulting in tubular dysfunction and inducing sodium retention and volume overload (Satarug et al., 2006), and oxidative stress damaging proteins and also reducing activity of Na^+/K^+ ATPase (an important membrane

protein involved in sodium transport located in the basolateral plasma membrane of the proximal tubule) (Thévenod & Friedmann, 1999).

A recent prospective cross-sectional study examined 50 client-owned senior cats (32 normotensive; average age 12.9 years and 18 hypertensive; average age 16 years) with renal function ranging from normal to moderately reduced, and explored the association of urinary cadmium excretion and hypertension in them. The results suggested an association between urinary cadmium excretion and hypertension in cats that is independent of renal function and with urinary cadmium concentration tending towards a significant correlation with age (Finch et al., 2012). Older cats have previously been reported to have significantly higher cadmium concentrations in kidney tissue than younger adult cats, suggesting chronic exposure (Andreani et al., 2010; Paßlack et al., 2014). The authors (Finch et al., 2012) concluded that the reported association between urinary cadmium excretion and hypertension suggested an effect of cumulative low-dose cadmium exposure on blood pressure in cats. However, they identified that further prospective studies were required to explore the mechanisms by which cadmium may contribute to development of hypertension offering important information into the pathophysiology of hypertension and opportunities for potential therapeutic intervention.

2.14 Conclusion

In conclusion, although some studies to date have identified cadmium accumulation in feline organs, the precise mechanisms of cadmium toxicity in cats are largely unknown. Given that many of the identified mechanisms/pathways, such as transport by MT, are present across multiple species it is likely that cadmium has the potential to be a causal factor of CKD in cats. Further consideration is required as to the dose of cadmium required to affect renal injury and whether the accumulation of cadmium will reach toxic levels. Cadmium contamination in cat food is of concern because it is a known toxicant that has the ability to cause renal injury, which is a key component in CKD, however, there is inadequate information regarding safe limits of cadmium in cat foods. Therefore, more investigation is required into the potential effects of cadmium in cats (such as renal damage) and the potential sources of exposure (such as dietary intake) in order to be able to prevent what may be cadmium-induced CKD.

2.15 Aims & hypothesis of this study

Testing of diets available in the marketplace (unpublished data), indicated that cats were potentially consuming >5x the maximum intake on a metabolic body weight basis by adult humans in regions of Europe with heavy industrial cadmium contamination of the environment (Panel on Contaminants in the Food Chain, 2009). Considering phosphate fertilisers continue to be a critical component of increasing productivity in New Zealand agriculture (Cavanagh et al., 2013) and soil mapping has revealed cadmium levels of 0.58 - 0.74mg/kg in some regions (Stafford et al., 2014) there is the potential for cattle in New Zealand to be consuming high levels of cadmium in their diets from fertilised pasture. The hypothesis examined in this study is that if cats are exposed to high levels of cadmium in their diets and that if they have similar mechanisms/pathways to that of humans, cadmium is a possible contributor to CKD, which has a high prevalence and largely unknown aetiology in cats. This study therefore aimed to examine the effect of high levels of cadmium in cat food on renal function in cats when compared to diets with lower levels of cadmium.

Chapter 3   Materials and methods

3.1   Animals

This study was carried out at the Feline Nutrition Unit of Massey University, Palmerston North, New Zealand from 10 October 2013 to 19 April 2014. The colony is a closed breeding unit used for nutritional research. Twenty seven domestic short hair cats (17 male and 10 female) were randomly selected from the colony population and assigned to 3 groups (n=9) which were balanced for age and sex as described in Table 3.1, a full breakdown is available in Appendix 1.1. The study was reviewed and approved by the Massey University Animal Ethics Committee (MUAEC protocol number 13/52).

Experimental Diet Group	Average Age at start of trial (years ±SD)	Gender distribution within groups
Low	6.73 ±2.53	6 Males 3 Females
Medium	6.92 ± 1.99	5 Males 4 Females
High	6.93 ± 1.99	6 Males 3 Females
Total	**6.86 ±2.10**	**17 Males** **10 Females**

Table 3.1 - Average age and gender distribution within the three experimental diet groups

3.2   Pre-trial conditioning and health screening

Having been born and raised in the Centre for Feline Nutrition the cats were familiar with the environment. In addition, as part of the management of the Centre all of these cats were trained to be handled, weighed and restrained for sampling procedures. Pre-trial blood screening was conducted to confirm that the cats were healthy prior to the trial starting, a complete blood count (CBC), feline biochemistry, and sick animal panel, were performed by a commercial diagnostic laboratory (New Zealand Veterinary Pathology, Palmerston North, New Zealand).

3.3   Pre-trial diet testing

3.3.1   Analysis of Cadmium in cat food

Three commercial meat-based cat foods, manufactured from New Zealand livestock by-products, were tested for their cadmium content to examine a representative sample of levels of cadmium that cats may ingest from their diet. The samples were crushed and homogenised prior to analysis for cadmium, following the methodology previously published (Jeyakumar et al., 2014) for plant metal analysis. Briefly, 0.1g of each sample was digested using 10mL 69% HNO_3 and stored for total cadmium concentration measurement. Cadmium in the diets was assessed using a Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS; PerkinElmer Analyst 600, PerkinElmer, Christchurch, New Zealand).

Testing established that the cadmium content of these diets ranged from 0.123mg Cd/kg to 0.520mg Cd/kg (Table 3.2); the ingredients that contained the highest cadmium content are beef kidneys, termed "low fat trim" (LFT) (A O'Rourke personal communication, July 7 2013). Thus, it can be seen that on a per kg basis, an average domestic cat in New Zealand consuming diets 1 and 3 will ingest 9.1-10.5µg Cd/kg BW/week, and a cat consuming diet 2 will ingest 39.55µg Cd/kg BW/week.

Table 3.2 - Proximate analysis (on a DM basis), estimated daily intake, and cadmium content of 3 canned cat foods. The daily intake is based on a 4kg cat with a maintenance energy requirement of 1038kJ.

Diet ID	Ash %	CP %	kJ/kg	Cd (mg/kg)	DM intake (g)	Cd intake (µg/day)	Daily intake (µg/kg BW)
1	6.2	48.5	2330	0.131	44.64	5.8	1.5
2	11.0	52.7	2390	0.520	43.40	22.6	5.65
3	9.6	56.1	2380	0.123	43.57	5.4	1.3

3.4   Experimental Diets and Food Intake

Cats were fed one of three canned experimental diets for 6 months based on the Chef Chunky Chicken recipe (Heinz Wattie's Ltd, Hastings, New Zealand) using standard raw ingredients with the amount of beef low fat trim (LFT), consisting of diaphragm muscle, smooth muscle surrounding the oesophagus, cheek and tongue tissue and kidneys, varied in order to generate the different cadmium levels (which were analysed after the trial period). Diets were manufactured in commercial facilities at Heinz Wattie's Ltd, Hastings, New Zealand.

Each group received one of the three experimental diets *ad libitum* over a 6-month period:

1. A low "low fat trim" diet (0% LFT). This contains the lowest concentration of cadmium that is possible using New Zealand-derived meat by-products.
2. A medium "low fat trim" diet (6% LFT).
3. A high "low fat trim" diet (30% LFT). This represents the highest reasonable concentration of cadmium content in New Zealand commercial or home-prepared cat foods.

All three diets were nutritionally complete and balanced and manufactured using conventional ingredients to meet or exceed the minimum AAFCO requirements for maintenance. The nutrient profiles are given in Table 3.3 and the list of dietary ingredients in Table 3.4.

Food was offered on a per pen (group) basis, once daily. Each cat was offered more than their daily requirement of 100 kcal/kg $BW^{0.67}$/day. Intake of food was then measured (on a per pen basis) by calculating the weight of the food offered, minus refusals collected and weighed the next morning. Access to food was therefore *ad libitum* except on iohexol sampling days. On experimental days' food was withdrawn on the morning of the trial, and then replaced on completion of collection of iohexol samples.

Food intake was then converted to 'mean week intake' to align with the weekly weighing of cats, then the amount of food per body weight calculated. This value was then converted to a DM basis and the mean weekly cadmium being consumed was then derived from this value.

Table 3.3 - Analysis of the composition of experimental diets on a DM basis

Diet Component	Diet		
	Low	Med	High
Moisture (%)	82.30	83.50	82.00
Crude Protein (% DM)	52.54	52.73	50.56
Crude Fat (% DM)	26.55	24.85	31.67
Carbohydrate (% DM)	6.78	8.48	6.11
Crude Fibre (% DM)	1.13	0.61	0.56
Ash (% DM)	12.99	10.91	8.89
Calcium (mg/kg DM)	3.00	2.91	2.28
Phosphorus (mg/kg DM)	1.79	1.33	1.00
Salt (% DM)	2.26	1.82	1.67
Metabolisable Energy (kJ/kg DM)	3200	2938	3525
Cadmium (µg/g DM)	0.062	0.027	0.461

Table 3.3 - Analysis of the composition of experimental diets on a DM basis

Table 3.4 - Dietary ingredients of the Low, Medium and High Cadmium diets

Ingredients	% in Diet		
	Low	Med	High
Beef lung lobes	21.74%	24.62%	9.23%
Chicken waste	24.84%	18.46%	12.31%
Chicken hash	4.66%	0%	0%
Chicken plucks	6.21%	0%	0%
Carrageenan Gum	0.75%	0.62%	0.62%
Corn Gluten	1.55%	1.54%	1.54%
Vitamin & mineral premix	0.05%	0.06%	0.06%
Water	39.27%	41.30%	38.23%
Beef liver	0%	6.15%	6.15%
Beef Low Fat Trim	0%	6.15%	30.77%
Locust bean gum	0%	0.12%	0.12%
Caramel liquid	0%	0.11%	0.11%
Sodium tripolyphosphate	0%	0.18%	0.18%
Shell powder	0%	0.55%	0.55%
Sodium nitrite	0%	0.003%	0.003%
Calcium hydroxide	0%	0.12%	0.12%

Table 3.4 - Dietary ingredients of the Low, Medium and High Cadmium diets

3.4.1 Palatability testing of experimental diets

Prior to study commencement the three experimental diets were tested for palatability using two palatability panels of colony cats different to that of the experimental groups. This testing was conducted using the standard acceptance testing methods used in the feline unit (Tarttelin, 1997). Two groups of 8 cats were trained to occupy small individual cages in an isolated room for a two-hour period over a five-day study period to test the palatability of diets. Cats were trained and selected to feed from small bowls without spillage and accept temporary isolation from their cage mates. Bowl positions were swapped each day to eliminate possible bias by cats by consistent left or right bowl preference. Analysis was by weight of food consumed expressed in three ways: 1. Preferred choice of diet (on the basis of the most eaten at each meal); 2. Mean intake of each diet over the five days of the test; and 3. Test of consistency of response of each cat over the five day period using weekly mean intake of each diet and calculation of the 95% confidence limits to measure the reliability of the mean (Tarttelin, 1997).

3.5 Trial Conditions

Cats were housed by experimental group in outdoor colony pens throughout the trial; the pens measured 2.4m high x 4.4m deep by 1.4m wide as pictured below in Figure 3.1. The cats were exposed to natural light cycles and provided with *ad libitum* access to water. During iohexol sampling cats were removed to a procedures room, following the completion of sample collection cats were returned to the group pen.

As a part of regular colony maintenance cats were weighed weekly and their weights recorded. This data was compared to the historical colony data for each individual to ensure that cats were not experiencing significant weight loss. Cats were withdrawn from the study if they lost more than 10% of BW compared with the weight recorded at the same week the previous year.

Figure 3.1 - A single Feline Centre colony pen.

3.6 Experimental design and timeline

Iohexol sampling was conducted at key study milestones (baseline (0 Months), 3 months and 6 months); meanwhile urinalysis and Complete Blood Count (CBC) blood analysis were conducted at monthly intervals throughout the study. A "Sick Animal" panel for biochemistry analysis was conducted at both baseline and 6 months; an experimental timeline of key events are presented in Figure 3.2 below and a more detailed table is provided in Appendix 1.2.

Figure 3.2 - Experimental timeline including weighing and sampling dates

Figure 3.2 - Experimental timeline including weighing and sampling dates

Timeline dates: 10 Oct, 20 Oct, 30 Oct, 9 Nov, 19 Nov, 29 Nov, 9 Dec, 19 Dec, 29 Dec, 8 Jan, 18 Jan, 28 Jan, 7 Feb, 17 Feb, 27 Feb, 9 Mar, 19 Mar, 29 Mar, 8 Apr, 18 Apr

Project Start — CBC, Biochemistry — Urinalysis, Iohexol, Weighed — Weighed
Diets started
Weighed
Weighed — CBC, Urinalysis
Weighed
Weighed
Weighed — CBC, Urinalysis
Weighed
Weighed
Weighed — CBC, Urinalysis — Urinalysis, Iohexol, Weighed
Weighed
Weighed
Weighed — CBC, Urinalysis
Weighed
Weighed — CBC, Urinalysis
Weighed
Weighed
Weighed, Blood Pressure
Weighed, Blood Pressure — CBC, Biochemistry, Urinalysis
Weighed, Blood Pressure — Urinalysis, Iohexol, Weighed
Project End

3.7   Blood collection

Cats were restrained in sternal recumbency with the neck outstretched and a jugular vein palpable. An area of approximately 5cm^2 over the jugular vein was clipped of hair and prepared aseptically for venepuncture using chlorhexidine surgical scrub and methylated spirits. A 3mL jugular venous blood sample was collected for CBC and biochemistry panel using a 25-gauge needle and 3mL syringe and then transferred into EDTA and heparin tubes. EDTA routine haematology (white blood cells (WBC), red blood cells (RBC), haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (PLAT), segmented neutrophils (SEGN), lymphocytes (LYMPH), monocytes (MONO), eosinophils (EOSIN), basophils (BASO)) and serum biochemistry (albumin : globulin ratio (AGR), albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate transaminase (AST), bilirubin (BIL), calcium (Ca), creatinine (CRE), cholesterol (CHOL), chloride (CL), creatine kinase (CK), globulins (GLO), potassium (K), phosphate (PO4), sodium (Na), total protein (TP), urea (URE)) were assayed by a commercial diagnostic laboratory (New Zealand Veterinary Pathology, Palmerston North, New Zealand) on the day of collection.

3.8   Iohexol administration and catheter placement

Assessment of renal function was completed using iohexol clearance (Von Hendy-Willson & Pressler, 2011). Cats were lightly restrained in sternal recumbency with the foreleg extended and a 22-gauge catheter (BD Insyte™, Becton Dickinson, Utah, USA) was placed into the cephalic vein. Blood was drawn back into the hub of the catheter to test patency before iohexol (50 mg/kg dose, using 300 mg/mL Omnipaque® x-ray-contrast solution: GE Healthcare, Auckland, New Zealand) was injected intravenously via the catheter, followed by a 1mL saline bolus (Baxter Healthcare Pty Ltd, Auckland, New Zealand) to ensure the full dose had been administered. The catheter was then removed. Total iohexol injection volumes were calculated on a bodyweight basis per cat.

Blood samples were collected by jugular venepuncture (as described in Section 3.7 above) at 2, 3, 4, and 5 hours post iohexol administration. A 1mL jugular venous blood sample was collected using a 25-gauge needle and 3mL syringe and transferred into a 6mL heparin vacutainer tube. The sample was then centrifuged at 14,000 x g for 10 minutes and the

serum pipetted into air-tight, plastic 1.5mL tab top microcentrifuge tubes (Raylab, Auckland, New Zealand) and stored at -80°C until assayed.

3.9   Urine Collection

Urine was collected by cystocentesis through the ventral abdominal midline, using a 25 gauge needle and 10 mL syringe. Cats were restrained by being held standing on their hind limbs and elevating the forequarters as described in the literature (Kurien et al., 2004). A small amount of the sample was used for an initial urinalysis using a digital refractometer to measure urine specific gravity (USG or SG) (UG-1 Urine S.G. Meter, Atago Co., Ltd, Tokyo, Japan) and dipstick (Combur[9] Test®, Roche Diagnostics Limited, Rotkreuz, Switzerland). Combur[9] Test® strips are capable of measuring; pH, leukocytes (LEU), nitrites (NIT), protein (PRO), Glucose (GLU), ketones (KET), urobilinogen (UBG), and bilirubin (BIL).

3.10 Blood Pressure

Cats were lightly restrained using the clipnosis technique of behavioural inhibition involving the placement of clips along the dorsal cervical region or "scruff" of the neck (Pozza et al., 2008). Blood pressure was then measured using a Pettrust Handheld Non-Invasive Blood Pressure Monitor (BioCARE Corporation, Taoyuan County, Taiwan). An appropriately sized cuff was selected for each animal using the marked index range on the inside of the cuff and then wrapped snugly around the foreleg leaving enough room between the cuff and the extremity for two fingers, as recommended by the manufacturer. The monitor was set to measure at 1 minute intervals and readings for systolic (Sys), diastolic (Dia), mean arterial pressure (MAP) in mmHg and pulse rate (bpm) were recorded for 3 consecutive cycles. Due to cat behaviour some measurements were taken using the method described above on the tail instead of the foreleg.

A baseline or 'normal' blood pressure was established by measuring all other adult cats in the colony (n=106) as described for the experimental cats. While cats in the same pen were measured in the same session, these measurements across the colony were conducted over a two-week period.

3.11 Analysis of Iohexol Concentrations in Blood Plasma

3.11.1 Sample preparation

Plasma samples were removed from the -80°C freezer and defrosted at room temperature. Micro-centrifuge tubes were then inverted to ensure the sample was completely mixed. 5µL of plasma was then diluted in 995µL of HPLC buffer solution (8 mM PO_4, pH 2.2, 10% MeOH) in 2mL clear combination seal vials (ThermoFisher Scientific, Auckland, New Zealand).

3.11.2 HPLC Analysis

The plasma clearance of iodine was taken as the measure of the administered dose of iohexol. Iohexol concentration in plasma was measured using a Shimadzu (Shimadzu Corporation, Kyoto, Japan) reverse-phase high performance liquid chromatography (RP-HPLC) system with a GraceSmart RP-C18 column, 4.6mm i.d., 250mm length, 5µ particle size, 120Å pore size (Grace, Maryland, USA). Samples were analysed by isocratic elution with Buffer A (8 mM PO4, pH 2.2, 10% MeOH) and Buffer B (80% CH_3CN), following methods previously published (Shihabi et al., 1993) with some modification.

The flow rate was maintained at 0.6mL/min. Two peaks corresponding to two isomers of iohexol eluted with UV detection at 254nm and the area under the second peak was measured. Concentration values were calculated using a comparison against known concentrations of iohexol constituting "standard curves" and calculating the "area under the curve" (AUC). Standard curves were calculated using iohexol diluted in HPLC buffer solution to 0, 0.5, 1.0, 2.5, 5.0, and 10µg/mL. Each sample was measured in quadruplicate, the mean area under the curve was calculated for each sample and the concentration calculated using the following formula: Mean AUC of the sample/Mean AUC calculated from the standard curve, and adjusted (x200) for the dilution factor.

3.12 Calculation of GFR

Iohexol plasma clearance was calculated using the following formula:

$$AUC_{t1-t2} = (t_2 - t_1) \times \frac{(C_1 - C_2)}{ln\left(\frac{C_1}{C_2}\right)}$$

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

Where t=time of blood collection after injection, c=concentration at that time, and ln = natural log. Thus the AUC is the sum of each of the trapezoids calculated between each collection time point. The clearance rate (cl) provides the rate in mL/min, which is then divided by body weight (BW) to give mL/kg/min.

3.13 Statistical Analysis

Data was analysed using a PROC Mixed repeated measure analysis in SAS v9.3 (SAS Institute Inc., Cary, North Carolina, USA) with diet and month as fixed effects in the model. Interactions between diet and month were assessed and discarded if not significant and the model was re-run with diet and month as single effects. The significance level was set at P= 0.05.

Chapter 4   Results

4.1   Animal Health

All animals remained healthy throughout the study (as assessed by physical examination and blood parameters), with the exception of Cat #1706 (Toko) which was diagnosed with an ear infection in January 2014 (week 13) and was treated daily for 8 days with Surolan (active ingredients Miconazole, polymyxin and prednisolone; Elanco Animal Health, Indianapolis, IN, USA), a combination antibiotic, antifungal, and corticosteroid ear drop.

4.1.1   Bodyweight and Food Intake

The mean weekly food and cadmium intake on a per kg body weight basis for the cats fed the low, medium and high cadmium diets is shown in Table 4.1. On average the cats ate between 10.31 - 15.41g/kg BW (on a DM basis) of food per week (raw intake is presented in Appendix 1.4). This translates to a weekly cadmium intake of 0.28ng/kg BW for cats fed the Medium diet, cats fed the Low diet had a 2.75 fold higher cadmium intake (0.77ng/kg BW), while cats fed the High diet (ng/kg BW) had 25.36 fold higher cadmium intake than the Medium group and 9.22 fold higher than the Low group as shown in Table 4.1.

Table 4.1 - Mean weekly food intake (g DM/kg BW) and cadmium (Cd) intake (ng DM/kg BW) ± Standard Error of the Mean (SEM) for cats fed the three experimental diets containing low, medium or high levels of cadmium over a 26 week period.

Intake Measure	Diets		
	Low	Med	High
Mean weekly food intake ±SEM (g DM/kg BW)	12.40 ± 0.16	10.31 ± 0.12	15.41 ± 0.29
Mean weekly food Cd intake ± SEM (ng DM/kg BW)	0.77 ± 0.01	0.28 ± 0.003	7.10 ± 0.13

All cats maintained a healthy body weight throughout the study (Appendix 1.5). Massey University Feline Unit policy is to withdraw any cat that loses more than 10% of their body weight when compared to colony records (unpublished data) for the same time the previous year; no individual cat was withdrawn from the study due to weight loss.

Regardless of the diet the cats were fed, all of the males in the study showed an overall trend of a gradual increase in BW (as shown in Figure 4.1). Similarly for the females fed the Low cadmium diet (as shown in Figure 4.1) there was an overall gradual, but constant increase in mean BW. In contrast the mean BW for females fed the Medium cadmium diet showed an overall gradual decrease, and for females fed the High cadmium diet a sharper overall decrease in the weight was observed over the study period.
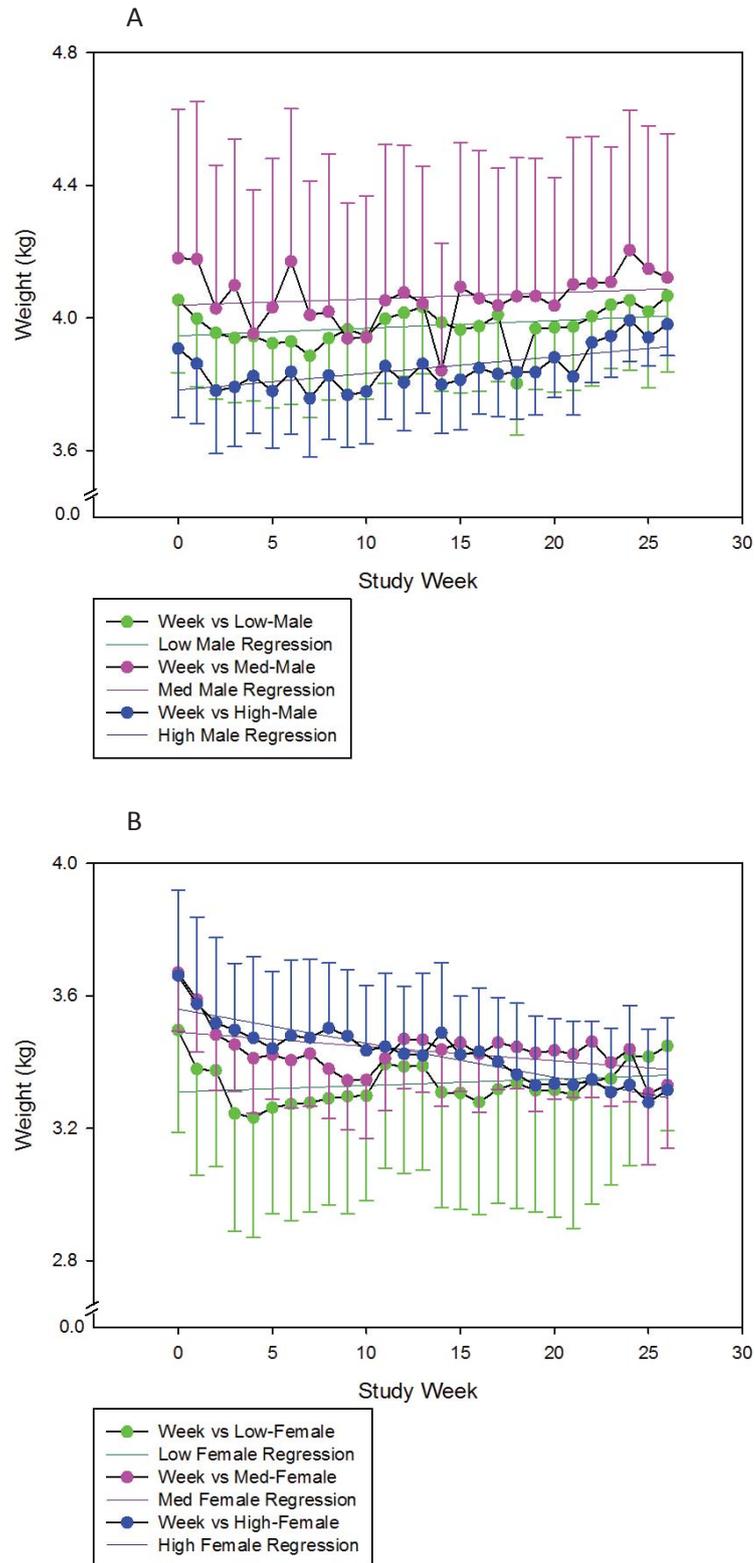
Figure 4.1 - Effect of dietary cadmium on mean weekly individual BW (kg) for cats (A: Males and B: Females) fed the three experimental diets containing low, medium or high levels of cadmium over a 26 week period.

4.1.2 Blood parameters

The effects of the level of cadmium in the diet on overall health of the cats over the 6 month period of the trial was assessed using a CBC and a Feline Sick Animal Panel measuring biochemical parameters (data presented in Table 4.2 and Table 4.3 respectively). On an individual level (as detailed in Appendix 1.6 and Appendix 1.7) there were variations in both haematological and biochemical values with some cats being slightly above or below the reference range. However once the data was combined all mean values were within the reference range, therefore significant differences at an individual level could be considered normal variation.

All haematological values were within the normal range for their respective measures. All values for banded neutrophils (BAND) were at or near the detection limit of the laboratory testing and therefore were not able to be analysed by the statistical software. Due to clumping in a number of the samples at Baseline, accurate platelet counts were not able to be reported, however the laboratory did report that numbers appeared to be adequate (i.e. were likely above the bottom of the normal range). Statistical significance was also not able to be determined in the relative cell volume of haematocrit, platelets and basophils due to the non-convergence of these values as these parameters are not always present or able to be counted in blood.

Red Blood Cell (RBC) numbers at the start and end of the study ranged from 8.42-8.61 $x10^{12}$/L for cats fed the Low cadmium diet, 8.48-8.91 $x10^{12}$/L for the Medium cadmium diet, and 8.86-9.34 $x10^{12}$/L fed the High cadmium diet, with the normal reported range being 5.0-10.0 $x10^{12}$/L (New Zealand Veterinary Pathology, 2014). There were no consistent diet differences and neither time, nor time x diet interactions were significant. At baseline, a number of artefactual abnormalities (due to sample handling) in red cell morphology were seen across all of the dietary groups including poikilocytosis, keratocytes and rouleaux in a number of samples, two samples individually showed evidence of Heinz bodies (Low cadmium diet) and Howell-Jolly bodies (High cadmium diet). The abnormal morphology was not present in any sample after the baseline readings.

At baseline, visual examination of samples detected reactive lymphocytes in the samples of one cat from each dietary group in the CBC by using a Romanowsky stain (M. Anderson, personal communication, August 11, 2016). No further reactive lymphocytes were detected at any other time point in the study.

Haemoglobin (Hb), mean corpuscular haemoglobin (MCH) and monocyte (MONO) numbers were statistically significant for time independent of diet or diet x time interactions. Mean corpuscular haemoglobin concentration (MCHC) was significant for diet (P= 0.03) and time (P=0.02) interactions individually but not diet x time interactions. Numbers of white blood cells (WBC), and the relative cell volume of Mean corpuscular volume (MCV), segmented neutrophils (SEGN), lymphocytes (LYMPH) and eosinophils (EOSIN) were not significant for diet, time or diet x time interactions.

Table 4.2 - Effects of the three experimental diets containing low, medium or high levels of cadmium on haematological values in cats fed over a 26 week period. Values are mean ± Standard Deviation

Measure	Reference Range	Month	Diets			Statistical Effect		
			Low	Med	High	Time	Diet	Time x Diet
White Blood Cells (WBC)	5.5-19.5 x10^9/L	Baseline	11.06 ±2.80	13.54 ±2.93	11.83 ±4.85	NS	NS	NS
		3 Months	12.17 ±2.85	12.34 ±3.41	14.07 ±5.29			
		6 Months	12.56 ±3.96	13.07 ±3.87	13.04 ±4.54			
Red Blood Cells (RBC)	5.0-10.0 x10^{12}/L	Baseline	8.42 ±0.42	8.91 ±0.58	9.34 ±0.88	NS	*	NS
		3 Months	8.61 ±0.69	8.48 ±0.64	9.01 ±0.72			
		6 Months	8.59 ±0.41	8.76 ±0.46	8.86 ±0.95			
Haemoglobin (Hb)	80-150 g/L	Baseline	119.89 ±3.59	127.78 ±13.10	130.56 ±11.73	*	NS	NS
		3 Months	120.00 ±10.50	119.33 ±14.08	124.89 ±9.60			
		6 Months	115.89 ±9.03	118.00 ±10.09	120.89 ±10.41			
Haematocrit (HCT)	0.24-0.45 L/L	Baseline	0.35 ±0.03	0.36 ±0.04	0.38 ±0.04	Did not converge	Did not converge	Did not converge
		3 Months	0.35 ±0.05	0.34 ±0.04	0.37 ±0.05			
		6 Months	0.35 ±0.04	0.34 ±0.03	0.37 ±0.05			
Mean corpuscular volume (MCV)	39-55 fL	Baseline	41.44 ±3.36	40.00 ±2.29	40.44 ±1.94	NS	NS	NS
		3 Months	40.44 ±4.53	39.56 ±2.01	41.33 ±4.58			
		6 Months	40.67 ±3.97	39.22 ±2.17	42.22 ±4.21			
Mean corpuscular haemoglobin (MCH)	13-18 pg	Baseline	14.44 ±0.73	14.33 ±0.87	14.11 ±0.78	*	NS	NS
		3 Months	14.00 ±1.00	14.11 ±0.78	14.00 ±0.87			
		6 Months	13.67 ±0.87	13.56 ±0.73	13.78 ±0.67			

Measure	Reference Range	Month	Diets			Statistical Effect		
			Low	Med	High	Time	Diet	Time x Diet
Mean corpuscular haemoglobin concentration (MCHC)	290-360 g/L	Baseline	346.56 ±20.35	345.33 ±38.01	348.56 ±11.20	*	*	NS
		3 Months	347.00 ±20.71	356.11 ±10.09	338.44 ±21.42			
		6 Months	333.44 ±17.69	344.44 ±8.96	326.56 ±22.19			
Platelets (PLAT)	300-800 x10^9/L	Baseline	NQ	NQ	NQ	Did not converge	Did not converge	Did not converge
		3 Months	293.03 ±296.48	347.00 ±102.57	171.67 ±155.54			
		6 Months	408.56 ±163.48	411.11 ±139.31	175.00 ±69.78			
Segmented neutrophils (SEGN)	2.4-12.5 x10^9/L	Baseline	6.06 ±1.72	9.26 ±2.87	7.15 ±4.54	NS	NS	NS
		3 Months	6.18 ±1.45	7.02 ±2.51	9.06 ±3.86			
		6 Months	6.97 ±2.55	7.92 ±2.76	7.95 ±3.34			
Lymphocytes (LYMPH)	1.5-7.0 x10^9/L	Baseline	3.78 ±1.27	3.32 ±1.35	3.94 ±1.44	NS	NS	NS
		3 Months	4.74 ±1.73	4.15 ±1.10	3.74 ±1.50			
		6 Months	3.68 ±1.67	3.74 ±1.54	3.42 ±1.28			
Monocytes (MONO)	0.0-0.9 x10^9/L	Baseline	0.34 ±0.31	0.27 ±0.18	0.32 ±0.25	*	NS	NS
		3 Months	0.37 ±0.10	0.35 ±0.15	0.39 ±0.21			
		6 Months	0.68 ±0.18	0.71 ±0.23	0.72 ±0.21			
Eosinophils (EOSIN)	0.0-1.5 x10^9/L	Baseline	0.72 ±0.43	0.65 ±0.28	0.70 ±0.44	NS	NS	NS
		3 Months	0.89 ±0.73	0.83 ±0.66	0.80 ±0.42			
		6 Months	1.23 ±1.15	0.69 ±0.40	0.96 ±0.69			
Basophils (BASO)	0.0 - 0.1 x10^9/L	Baseline	0.17 ±0.28	0.09 ±0.05	0.06 ±0.12	Did not converge	Did not converge	Did not converge
		3 Months	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00			
		6 Months	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00			

NS = not significant, *= P<0.05, NQ = Not able to quantify due to clumping of sample, SD = Standard Deviation.

The effects of cadmium exposure in the diet on serum biochemical parameters are displayed in Table 4.3. All values were within the normal range for their respective measures. All bilirubin values were at, or near the detection limit of the laboratory assays so were therefore unable to be analysed using the statistical software.

There were no statistically significant differences for diet, time, or diet x time interactions for the following measures: albumin: globulin ratio, albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), calcium, cholesterol, creatine kinase (CK), and potassium. Time was a significant factor for aspartate transaminase (AST), chloride (CL), globulins (GLO), total protein and sodium, but neither diet, nor diet x time interactions were significant.

The effect of cadmium exposure in the diet on serum creatinine (CRE) was significant (P= 0.01) for diet, but not time or diet x time interactions. While some changes were observed in creatinine values across the study, these were not significant, and the serum creatinine remained within the normal range (70 - 159μmol/L, New Zealand Veterinary Pathology, 2014).

The effect of cadmium exposure in the diet on serum total protein (TP) was statistically significant (P= 0.002) for time, but neither diet nor diet x time interactions were significant. Between baseline and 6 months' total protein (range 63 – 83g/L, New Zealand Veterinary Pathology, 2014) decreased 4.66g/L in cats fed the Low cadmium diet, 5.01g/L in cats fed the Medium cadmium diet and 4.89g/L in cats fed the High cadmium diet.

The effect cadmium exposure in the diet on serum phosphate was not significant for diet or time; however, there was a significant (P= 0.01) diet x time interaction. Phosphate concentrations were significantly different between baseline and 6 months for cats fed the Low and the Medium cadmium diets. At 6 months' serum phosphate (range 1.30 - 2.80mmol/L, New Zealand Veterinary Pathology, 2014) for cats fed the Low cadmium diet was 0.2mmol/L higher than at Baseline (P=0.03) and for cats fed the Medium cadmium diet was 0.2mmol/L lower at 6 months than at Baseline (P=0.03). For cats fed the High cadmium diet the serum phosphate concentration was not significantly different between baseline and 6 months. Comparing between groups there were no significant differences at baseline, however at 6 months' cats fed the Medium cadmium diet had significantly lower serum phosphate to both the cats fed the High cadmium diet (0.22mmol/L) and Low cadmium diet (0.29mmol/L), which were not significantly different to each other.

The effect of cadmium exposure in the diet on serum urea was not significant for diet interactions individually; however, both time (P= 0.04) and diet x time (P= 0.01) interactions were significant. The serum urea levels (range 5.7 - 12.9mmol/L, New Zealand Veterinary Pathology, 2014) in the cats fed the High cadmium diet rose by 0.71mmol/L between baseline and 6 months but were not significantly different. Urea levels in the cats fed the Low cadmium diet between baseline and 6 months dropped 0.98mmol/L which, while not significant (P=0.06), does represent a trend. For cats fed the Medium cadmium diet, urea levels dropped 1.54mmol/L between baseline and 6 months, which was the only group to show a significant difference between the start and end of the trial. There were no significant differences in serum urea between all groups at baseline, however at the conclusion of the trial cats fed the High diet had significantly higher urea levels compared to those fed both the Medium (by 1.7mmol/L) and Low (by 1.38mmol/L) cadmium diets.

Table 4.3 - Effects of dietary cadmium levels on serum biochemistry values of cats as measured by a feline sick animal panel at the start of the study and after 6 months of feeding the three experimental diets containing low, medium or high levels of cadmium. Values are presented as mean ± Standard Deviation.

Measure	Reference Range	Month	Diet			Statistical Effect		
			Low	Med	High	Time	Diet	Time x Diet
Albumin :Globulin Ratio (AGR)	0.6 - 1.6	Baseline	0.88 ±0.16	0.83 ±0.16	0.74 ±0.10	NS	NS	NS
		6 Months	0.94 ±0.15	0.79 ±0.19	0.86 ±0.10			
Albumin (ALB)	28 – 42 g/L	Baseline	34.78 ±1.99	35.22 ±2.77	33.44 ±2.13	NS	NS	NS
		6 Months	33.89 ±2.47	31.80 ±4.66	33.67 ±2.50			
Alkaline phosphatase (ALP)	0 – 85 IU/L	Baseline	24.67 ±6.91	27.33 ±7.18	24.00 ±4.12	NS	NS	NS
		6 Months	27.00 ±11.26	22.80 ±11.42	23.67 ±5.59			
Alanine Aminotransferase (ALT)	0 - 100 IU/L	Baseline	43.33 ±13.10	47.56 ±9.88	45.22 ±9.20	NS	NS	NS
		6 Months	48.22 ±10.81	41.90 ±11.49	57.33 ±14.71			
Aspartate transaminase (AST)	0 - 66 IU/L	Baseline	21.67 ±6.32	23.00 ±9.31	22.22 ±4.18	*	NS	NS
		6 Months	32.33 ±9.25	26.00 ±3.42	32.33 ±8.22			
Bilirubin (BIL)	0 - 5 mmol/L	Baseline	BDL	BDL	BDL	BDL	BDL	BDL
		6 Months	BDL	BDL	BDL			
Calcium (Ca)	1.81 - 2.70 mmol/L	Baseline	2.44 ±0.36	2.34 ±0.13	2.28 ±0.06	NS	NS	NS
		6 Months	2.33 ±0.12	2.30 ±0.15	2.29 ±0.09			
Creatinine (CRE)	70 - 159 µmol/L	Baseline	97.33 ±13.82	109.56 ±13.49	106.67 ±16.27	NS	*	NS
		6 Months	95.33 ±15.11	111.20 ±18.91	94.56 ±11.18			
Cholesterol (CHOL)	1.5 - 6.0 mmol/L	Baseline	3.94 ±0.86	3.40 ±0.76	3.33 ±0.72	NS	NS	NS
		6 Months	3.42 ±0.80	3.24 ±1.00	3.57 ±1.05			
Chloride (CL)	108 – 128 mmol/L	Baseline	115.11 ±1.69	114.67 ±3.00	113.78 ±1.39	*	NS	NS
		6 Months	118.33 ±2.12	118.90 ±1.32	117.78 ±1.64			

Measure	Reference Range	Month	Diet			Statistical Effect		
			Low	Med	High	Time	Diet	Time x Diet
Creatine kinase (CK)	0 - 344 IU/L	Baseline	193.00 ±96.28	209.44 ±119.46	188.33 ±74.80	NS	NS	NS
		6 Months	246.56 ±98.13	175.20 ±29.78	278.00 ±132.50			
Globulins (GLO)	27 – 49 g/L	Baseline	40.56 ±5.88	42.89 ±6.43	44.67 ±3.32	*	NS	NS
		6 Months	36.78 ±6.00	41.30 ±6.63	39.56 ±4.28			
Potassium (K)	3.5 - 5.0 mmol/L	Baseline	4.61 ±0.44	4.61 ±0.39	4.71 ±0.28	NS	NS	NS
		6 Months	4.96 ±0.49	4.79 ±0.40	4.77 ±0.19			
Phosphate (PO$_4$)	1.30 - 2.80 mmol/L	Baseline	1.45± 0.22[a]	1.56 ±0.25[a]	1.53 ±0.13	NS	NS	*
		6 Months	1.65± 0.23[b,x]	1.36 ±0.14[b,y]	1.58 ±0.14[x]			
Sodium (Na)	147 – 156 mmol/L	Baseline	153.89 ±1.83	154.11 ±3.22	152.78 ±0.83	*	NS	NS
		6 Months	151.89 ±1.62	151.40 ±1.13	151.67 ±0.87			
Total Protein (TP)	63 – 83 g/L	Baseline	75.33 ±4.56	78.11 ±5.40	78.11 ±3.37	*	NS	NS
		6 Months	70.67 ±6.56	73.10 ±7.79	73.22 ±5.85			
Urea (URE)	5.7 - 12.9 mmol/L	Baseline	8.92 ±0.87	9.16 ±1.18[a]	8.61 ±0.90	*	NS	*
		6 Months	7.94± 1.17[y]	7.62 ±1.04[b,y]	9.32 ±1.21[x]			

BDL = at/below detection limit, NS = not significant, *= P<0.05. Means with different superscripts within column (ab) and within row (xy) are significantly different (P<0.05).

4.1.3   Urine parameters

The effect of dietary exposure to cadmium on urine specific gravity (USG) is shown in Table 4.4 (Archer, 2005). The normal reference range for feline USG is 1.035 - 1.060 (Archer, 2005). A more dilute urine (< 1.035) may represent poorly concentrated urine, whereas at the higher end of the range (>1.060) represents a highly concentrated urine and may indicate dehydration (Archer, 2005). On an individual level (as detailed in Appendix 1.8) there were variations in USG values, with some cats being slightly above or below the reference range, on a group basis all values were within the lower half of the normal reference range, ranging between 1.02-1.04. All USG measures in cats fed the Low and High cadmium diets were consistent throughout the study as shown in Table 4.4. Also USG for all

cats fed the Medium cadmium diet were similar to cats fed both the Low and High cadmium diets at baseline, 2, 3, and 5 months, and a USG within 0.01 of the other groups at 4 and 6 months.

The effect of the level of cadmium in the diet on urinary pH is also shown in Table 4.4. On an individual level (as detailed in Appendix 1.8) there were variations in pH values with some cats being slightly above or below the reference range (6.0 – 7.5 (Archer, 2005)), all values were also within the normal feline reference range with most values tending towards the lower end of the range. Mean urinary pH values ranged from 6.00 to 7.00; within groups the pH variation (between the highest and lowest value) was 0.61 for cats fed the Low cadmium diet, 0.94 for cats fed the Medium cadmium diet, and 0.72 for cats fed the High cadmium diet. Variations in urinary pH (between the highest and lowest value) across the groups at the key milestones of the study were; at baseline 1.0, at 3 months 0.67, and at 6 months 0.44.

Table 4.4 - Effects of dietary cadmium levels on Urinary specific gravity and pH for cats fed the three experimental diets containing low, medium or high levels of cadmium over a 26 week period. Values are presented as mean ± standard error.

Measure	Reference Range	Month	Diet		
			Low	Med	High
Urine Specific Gravity (USG)	$1.035 - 1.060$[1]	Baseline	1.03 ±0.004	1.03 ±0.004	1.03 ±0.004
		2	1.04 ±0.003	1.04 ±0.003	1.04 ±0.003
		3	1.04 ±0.003	1.04 ±0.003	1.04 ±0.003
		4	1.03 ±0.003	1.04 ±0.003	1.03 ±0.003
		5	1.04 ±0.003	1.04 ±0.003	1.04 ±0.003
		6	1.03 ±0.003	1.02 ±0.003	1.03 ±0.003
Urinary pH	$6.0 - 7.5$[1]	Baseline	6.67 ±0.28	6.00 ±0.28	7.00 ±0.28
		3	6.22 ±0.23	6.89 ±0.23	6.72 ±0.23
		4	6.44 ±0.24	6.67 ±0.24	6.28 ±0.24
		5	6.39 ±0.23	6.94 ±0.23	6.56 ±0.23
		6	6.83 ±0.32	6.88 ±0.32	6.44 ±0.32

[1] **(Archer, 2005).**

In addition to pH the urine dipstick analysis detected some transient changes in erythrocytes (ERY) and haemoglobin (Hb) as summarised in Table 4.5, full data on all measures is presented in Appendix 1.8. Glucose (GLU), ketones (KET), nitrites (NIT), urobilinogen (UBG), and bilirubin (BIL), were all negative throughout the study. Leukocytes (LEU) were excluded as they are considered to be unreliable in cats (Callens & Bartges, 2015; Reppas & Foster, 2016).

Protein (summarised in Table 4.5) was detected in a single sample at baseline from a cat fed the Low diet. Between months 3-6 protein was consistently detected at low (+1) levels in all dietary groups, with the exception of cats fed the High diet at 3 months. At this time two cats fed the High diet has a reading of +2 and another cat had a reading of +3, each of these individuals returned to either low (+1) or Nil levels of protein the following month.

Table 4.5 - Urinary dipstick protein, haemoglobin and erythrocyte measures within the Low, Medium and High cadmium dietary groups at 0, 3, and 4-6 months

Measure	Month	Diet		
		Low	Med	High
Protein	0	1x +1	Nil	Nil
	2	No data	No data	No data
	3	5x +1	5x +1	6x +1 2x +2 1x +3
	4	7x +1	8x +1	8x +1
	5	5x +1	7x +1	4x +1
	6	4x +1	4x +1	4x +1
Haemoglobin	0	Nil	Nil	Nil
	2	No data	No data	No data
	3	1x +3	Nil	Nil
	4	2x +1 1x +3	1x +2	1x +1 1x +2
	5	1x +2 1x +3	Nil	Nil
	6	1x +3	Nil	Nil
Erythrocytes	0	Nil	Nil	Nil
	2	No data	No data	No data
	3	Nil	Nil	Nil
	4	Nil	Nil	Nil
	5	Nil	Nil	Nil
	6	Nil	2x +3	Nil

4.1.4 Blood Pressure

Blood pressure was measured to examine the potential link between hypertension and kidney disease between the groups following 6 months of feeding. Given that the blood pressures were highly variable and overlapped it was clear there was no difference (P>0.05).

The effect of dietary exposure to cadmium on blood pressure in cats measured over the last 3 weeks of the study is shown in Table 4.6 and individual values are available in Appendix 1.9. Mean Systolic Blood Pressure (SBP) values were all within the normal feline reference range (110.0–180.0mmHg (Lin et al., 2006)), cats fed the Low cadmium diet ranged between 128.0 – 155.4mmHg, cats fed the Medium cadmium diet 135.6 – 148.8mmHg and cats fed the High cadmium diet 130.9 - 148.0mmHg.

There are no individual reference values available for Diastolic Blood Pressure (DBP), as hypertension (high blood pressure) (Brown et al., 2007), and the staging of CKD (Bartges, 2012) is diagnosed in conjunction with SBP rather than DBP alone. Mean DBP ranged from 92.1 – 105.0mmHg in cats fed the Low cadmium diet, 79.7 - 105.3 for cats fed the Medium cadmium diet, and 86.0 - 109.4mmHg for cats fed the High cadmium diet (Table 4.6).

For Mean Arterial Pressure (MAP) no reference values were able to be obtained from the literature. As shown in Table 4.6, mean values ranged from 107.1 - 122.0mmHg for cats fed the Low cadmium diet, 98.3 – 116. 7mmHg for cats fed the Medium cadmium diet and 102.1 – 122.4mmHg for cats fed the High cadmium diet.

All heart rates, as shown in Table 4.6, were also within the normal reference range. Within groups mean BPM varied across the 3 days measured (between lowest and highest value) by 6 BPM for cats fed the Low cadmium diet, 16.9 BPM for cats fed the Medium cadmium diet, and 30.9 BPM for cats fed the High cadmium diet.

Table 4.6 - Effects of dietary cadmium levels on blood pressure for cats fed the three experimental diets containing low, medium or high levels of cadmium over a 26 week period. Values are presented as mean ± standard error.

Measure	Reference Range	Study Day	Diet		
			Low	Med	High
Systolic (SBP)	110.0–180.0mmHg[1]	167	128.00 ±7.45	148.78 ±7.45	138.11 ±7.45
		174	155.44 ±6.85	139.78 ±6.85	130.89 ±6.85
		181	138.44 ±7.56	135.56 ±7.56	148.00 ±7.56
Diastolic (DBP)	n/a	167	96.78 ±6.71	97.33 ±6.71	86.00 ±6.71
		174	105.00 ±6.24	105.33 ±6.24	87.67 ±6.24
		181	92.11 ±6.85	79.67 ±6.85	109.44 ±6.85
Mean Arterial Pressure (MAP)	n/a	167	107.11 ±5.97	114.22 ±5.97	103.00 ±5.97
		174	122.00 ±5.88	116.67 ±5.88	102.11 ±5.88
		181	107.56 ±6.55	98.33 ±6.55	122.44 ±6.55
Heart Rate	110-180[2] (Beats per minute - BPM)	167	134.89 ±8.79	145.78 ±8.79	103.44 ±8.79
		174	136.67 ±9.22	130.56 ±9.22	134.33 ±9.22
		181	128.89 ±6.44	128.89 ±6.44	121.33 ±6.44

[1](Lin et al., 2006), [2](Howell, 2007).

4.2 GFR

A 'normal' GFR is poorly defined in cats, and a definitive reference range has not been agreed upon (Von Hendy-Willson & Pressler, 2011), however using the most similar technique to that used in the current study (Heiene et al., 2009) a range of 1.02 - 3.50mL/min/kg with a mean GFR of 2.26 ±0.62 mL/min/kg could be considered as a reference point. Iodine concentrations and times used to calculate the individual GFRs are available in Appendix 1.10 and Appendix 1.3 respectively and the average group GFRs are presented in Table 4.7.

GFR's for cats fed the Low cadmium diet, over the 6-month trial period, ranged from 0.88-5.11mL/min/kg (median 2.74 mL/min/kg) at Baseline, 1.48 - 3.45mL/min/kg (median 2.31mL/min/kg) at 3 months, and 1.82 - 5.91mL/min/kg (median 2.79mL/min/kg) at 6 months. Within the Low group mean GFRs, at Baseline (2.72mL/min/kg) were not significantly different to either 3 months (2.17 mL/min/kg), or 6 months (3.34mL/min/kg), however GFR at 3 months was significantly lower than at 6 months (P<0.05).

GFR's for cats fed the Medium cadmium diet, over the 6-month trial period, ranged from 1.24 - 2.76mL/min/kg (median 1.76mL/min/kg) at the baseline, 1.52 - 4.58mL/min/kg (median 3.74mL/min/kg) at 3 months, and 1.56 - 3.66mL/min/kg (median 2.04mL/min/kg) at 6 months. Within the Medium cadmium diet group mean GFR, as shown in Table 4.7, was significantly lower at baseline (1.83mL/min/kg) than at 3 months (3.16mL/min/kg) but at 6 months the GFR (2.45mL/min/kg) was not significantly different to either of the previous time points.

GFRs for cats fed the High cadmium diet, over the 6-month trial period, ranged from 1.32 - 3.25mL/min/kg (median 2.49mL/min/kg) at baseline, 1.72 - 5.63mL/min/kg (median 3.38mL/min/kg) at 3 months, and 1.67 - 3.78mL/min/kg (median 3.20mL/min/kg) at 6 months. Within the High cadmium diet group, as shown in Table 4.7 mean GFR was significantly lower at baseline (2.49 mL/min/kg) compared to 3 months (3.49 mL/min/kg) but at 6 months the GFR (2.94 mL/min/kg) was not significantly different to either of the previous time points.

At baseline, GFRs in cats fed the Low cadmium diet were significantly higher than those on Medium cadmium diet; however, cats fed the High cadmium diet were not significantly different to cats in either the Low or Medium groups. At 3 months' cats fed the High cadmium diet had significantly higher GFRs than cats fed the Low cadmium diet, while cats fed the Medium cadmium diet weren't significantly different to either group, although while not significant (P=0.07), a trend can be seen between cats fed the Medium and Low cadmium diets. By the end of the trial at 6 months none of the diet groups were significantly different to the others.

Table 4.7 - Mean Glomerular Filtration Rate ± Standard Error for cats fed the three experimental diets containing low, medium or high levels of cadmium over a 26 week period.

Month	Diet			Statistical Effect		
	Low	Med	High	Time	Diet	Time x Diet
Baseline	2.72 ±0.29^{ab,y}	1.83 ±0.29^{a,x}	2.49 ±0.29^{a,xy}	*	NS	*
3 Months	2.17 ±0.38^{a,y}	3.16 ±0.38^{b,xy}	3.49 ±0.38^{b,x}			
6 Months	3.34 ±0.34^{b}	2.45 ±0.34^{ab}	2.94 ±0.34^{ab}			

NS = not significant (P>0.05), * = P<0.05. Means with different superscripts within column (ab) and within row (xy) are significantly different (P<0.05).

Chapter 5 Discussion

As discussed in Sections 2.9 and 2.12 cadmium is an impurity that is naturally present in phosphate rock, and consequently is present in all phosphate fertilisers (Cadmium Working Group, 2008; Stafford et al., 2014) which are a critical component of increasing productivity in New Zealand agriculture (Cavanagh et al., 2013). Potentially toxic compounds (such as cadmium) can then be taken up plants used for feed production (van der Fels-Klerx et al., 2011) and accumulate in consumable animal products, such as liver, kidney, and meat (Satarug et al., 2006; Paßlack et al., 2014). Because of the requirement for meat in feline diets (see Section 2.1.2) , and the high levels of meat by-products and offal utilised by pet food manufacturers in their diets, cadmium has the potential to affect cats and may be a causal factor in Chronic Kidney Disease (CKD) as outlined in Section 2.13.

5.1 Dietary Cadmium Levels and potential mechanisms of renal injury

In the current study, cats were fed diets designed to represent the potential range of cadmium that cats may be exposed to on wet diets in New Zealand. The manufacturing of the diets calculated that cadmium, on a weekly intake per kg body weight (BW) basis, ranged from 0.77 – 7.10ng. Accordingly, the separation of the Medium diet was much closer to the Low dietary group (2.75 fold higher) cadmium intake than to the High dietary group (51.23 fold higher cadmium intake than in cats fed the Low diet and 23.34 fold higher than cats the Medium diet). Although the raw material was sourced from cows in the same region, it is likely there are large differences in fertiliser use between farms and therefore cadmium contamination of the pasture. Low Fat Trim (LFT) (which contains a significant proportion of organ meat) is the dietary ingredient responsible for the cadmium contamination, adding more LFT to the Medium diet compared to the Low diet did not result in higher cadmium levels which would suggest that either; small amounts of LFT in the Low diet contained high levels of cadmium, or the higher LFT amounts in the Medium diet contained lower cadmium levels, or both.

Diets for this study had maximum cadmium levels of 0.0071µg/g DM, which is lower than reported in a previous overseas study that examined levels of cadmium in three wet cat foods and reported cadmium levels between 0.94 - 2.14µg/g on a DM basis (Duran et al., 2010). This suggests that in the current study, because diets were made from the same batch of ingredients, the bulking of raw material may have diluted cadmium levels. The key meat ingredient (beef, fish, or chicken) in each of the foods in the previous work had been sourced from different countries, with one of the 15 diets examined (a dry dog food, with beef as the key ingredient) sourced from New Zealand which had the second lowest level of cadmium contamination (0.75µg/g DM). This further suggests the level of risk associated with cadmium intake is not only specific to the area/region in which the ingredients for the cat food are sourced from, but also the country.

Duran (2010) also found that fish as the key meat ingredient contained the highest cadmium contamination with an average level of 1.89µg/g DM compared with 1.39µg/g DM in beef, and 1.19µg/g DM in chicken, across all cat and dog foods analysed. Wastewater discharge and surface runoff are important sources of contaminants that reach coastal waters due to anthropogenic activities (Oursel et al., 2014). Phaeophytae (brown algae) is the largest marine macrophyte (aquatic plant) metal accumulator and can accumulate elevated concentrations of trace metals in their tissues, including cadmium (Sánchez-Quiles et al., 2017). It is generally accepted that the metal load in the body of a fish is strongly affected by feeding behaviour and plays an important role in the metal content of tissues (Storelli & Marcotrigiano, 2004).

With increasing variety in and the convenience of prepared pet foods (Bontempo, 2005) a huge number of different diet combinations are fed (Laflamme et al., 2008). Considering that some foods may contain a higher cadmium component than others suggests that pet cats are likely to be eating diets of varying cadmium levels and these may either subsequently provide an extra safety margin or, conversely some cats may be at a higher risk than others, due to location or type of the key ingredient used in the food.

Analysis of three commercial diets on the New Zealand market prior to the start of the main study found that on a per kg basis, a cat consuming only these diets would ingest between 9.1µg and 39.55µg/Cd/week. On a metabolic body weight basis, the highest dose represents more than five times the maximum intake by adult humans in regions of Europe with heavy industrial cadmium contamination of the environment (Panel on Contaminants in the Food Chain, 2009). The only available information regarding the effects of dietary

cadmium in cats, reported that feeding 100mg Cd/day for one month resulted in varying degrees of desquamation in the proximal tubular epithelium (Prodan (1932) as cited in International Programme on Chemical Safety, 1992); in comparison, cats fed the diet with the highest cadmium level in the current study and similarly the highest commercial diet analysed in the current study, would receive a small fraction (0.000007% and 0.0004% respectively) of the cadmium dose Prodan (1932) used.

For humans (as discussed in section 2.11), the Agency for Toxic Substances and Disease Registry (ATSDR - a federal public health agency within the United States Department of Health and Human Services) derived a recommended maximum total daily intake (TDI) of 0.1µg/Cd/kg BW (equivalent to 0.7µg/Cd/kg BW/week) (Ministry for the Environment, 2011); while the Europe Food Safety Authority (EFSA) have derived a provisional tolerable weekly intake (PTWI) for humans of 2.5µg/Cd/kg BW (Panel on Contaminants in the Food Chain, 2009) and JECFA (an international scientific expert committee jointly administered by the Food and Agriculture Organization of the United Nations and the WHO) have recommended a PTWI of 0.8µg/Cd/kg BW/day (5.68µg/Cd/kg BW/week) (Ministry for the Environment, 2011). In comparison, in the current study a weekly cadmium intake (on a DM basis) of 39.45µg/kg BW for cats fed the highest cadmium diet represents a level 7 times higher than the least conservative value, or 56 times higher than the most conservative of the human intake guidelines.

In humans (see Sections 2.10.5 and 2.11), cadmium absorption after dietary exposure is relatively low (3–5%), but the cadmium that is absorbed, is retained in the kidney and liver. Both organs store between 50–85% of the body burden, with 30–60% of this being stored in the kidneys (World Health Organization, 2011), although liver stores are gradually released into the blood and are redistributed to the kidneys through circulation (Dudley et al., 1985). However, even low exposure levels may, over time, cause accumulation; especially in the kidneys as cadmium has a very long biological half-life ranging from 10 to 30 years (World Health Organization, 2011).

As discussed in Sections 2.10.5 and 2.11 Metallothionein (MT) is an abundant intracellular protein in the animal kingdom, although in higher organisms it can also occur in extracellular space such as blood plasma and cerebrospinal fluid (Sigel et al., 2013). Its physiological functions are not fully elucidated (Klaassen et al., 1999); however, given its high affinity for divalent heavy metal ions, MT seems to play an important role in regulating metal homeostasis (Vašák, 2005). Several human and rodent studies have suggested that

MT plays an important role in cadmium disposition and detoxification, and it has been used in a number of studies as a biomarker of both cadmium exposure and of cadmium induced proximal tubular injury (Klaassen et al., 1999; Prozialeck & Edwards, 2010).

In humans, there is a large amount of individual variation in MT expression, possibly due to polymorphisms (Wu et al., 2000), although similar work does not appear to have been conducted in cats. Polymorphisms in the human MT-2A gene can limit MT expression, increasing the susceptibility to cadmium toxicity and may be responsible for inter-individual differences in predispositions to cadmium toxicity (Kita et al., 2006; Kayaalti et al., 2010; Kayaalti et al., 2011).

There is emerging evidence suggesting that cats exhibit genetic and functional differences in detoxification pathways when compared with other species. Although largely attributed to drug metabolism pathways (Court & Greenblatt, 2000; Mealey, 2012; Court, 2013), the principle may be applicable for cadmium detoxification pathways as well. However, to assess the effects and toxicities of xenobiotics, it is necessary to identify the isoforms responsible for the metabolism in each species (Kobayashi et al., 2002).

The ATP-binding cassette (ABC) transporters are a large family of trans-membrane proteins that transport various molecules across cell membranes. As discussed in Section 2.10.5, cadmium is thought to be capable of binding to, and interfering with, the ABC family of detoxification transporter pumps which may be a mechanism of cadmium transport (Sigel et al., 2013). Much of the information on ABC transporters has been derived from human and rodent studies and while it is thought to be applicable to other mammalian species it has not be investigated directly in cats (Mealey, 2012).

The ABC transporter family contains over 40 members divided into seven subfamilies designated ABC 'A' through 'G' (Dean & Annilo, 2005). While ABCG2 is starting to be realised as of pharmacological importance in veterinary medicine (Mealey, 2012), ABCB1, C2, and C7, have been implicated as potential mechanisms in cadmium toxicity pathways (Sigel et al., 2013).

Like other ABC transporters, ABCG2 pumps substrates from the cytoplasm out of the cell, protecting the host from potential toxicity (Mealey, 2012). This is achieved by transporting substrate drugs from the hepatocyte to bile, from intestinal epithelial cells to the lumen of the intestine, and from brain or retinal endothelial cells to the capillary lumen, restricting access of potential toxins (Jonker et al., 2005; Mealey, 2012).

Several polymorphisms in ABCG2 have been defined in humans and have been shown to decrease ABCG2 expression and /or reduce ABCG2 transport activity (Mealey, 2012). One study has found feline-specific changes in ABCG2 in 100% of the 64 cats sequenced; more specifically, three of these amino acid changes are unique to the cat compared with 10 other mammalian species. Because these changes occur in highly conserved areas, they are expected to result in functional alterations of feline ABCG2 (Ramirez et al., 2011). It is therefore important to consider species specific differences in detoxification pathways which may render assumptions in humans, such as cadmium induced kidney disease, invalid in cats. More research is needed into cadmium detoxification pathways and potential functional alterations due to polymorphisms in key transport mechanisms in cats.

The effects of cadmium are thought to only be apparent only at high concentrations and thus *in vitro* experiments that utilise high concentrations to define mechanisms may have limited, if any, relevance for the lower tissue concentrations that are achieved *in vivo* (Sigel et al., 2013). Little evidence is available on the "natural" cadmium levels in the organs of cats (Paßlack et al., 2014), and how this relates to potential pathologies, particularly of the kidneys and/or liver (Andreani et al., 2010; Finch et al., 2012; Paßlack et al., 2014). It is important to evaluate if the accumulation of cadmium is dependent on kidney function and in particular, whether disorders in the renal excretory mechanisms either increase or decrease cadmium deposition in the kidneys of cats (Paßlack et al., 2014).

5.2   Bodyweight and Food Intake

Weight loss in cats with CKD is commonly reported with a prevalence of between 42–82% (Freeman et al., 2016). Weight loss is an important aspect in relation to CKD because it can negatively impact survival time via either direct effects of weight and muscle loss (e.g. weakness and impaired immune function), or indirectly through owners choosing euthanasia (often due to a combination of weight loss and decreased appetite). More importantly weight loss can be used as an early indicator for CKD in cats before diagnosis by techniques such as serum creatinine (Freeman et al., 2016). Therefore, in the current study, the use of historical data from colony records allowed cats to be individually matched to their historical data to assess their health while allowing for seasonal variation in bodyweight.

It is important to consider seasonal variation in body weight in cats. Lean mass has been shown to be affected by season, possibly due to seasonal differences in nutrient

digestibility (Bermingham et al., 2013). In the current study there was an initial general trend of bodyweight loss associated with summer in all dietary groups, while towards the end of the study the males showed an increase in weight while the females did not. One interesting finding was that the downward trend of the weight in females was more pronounced in those consuming the High cadmium diet. This may just indicate a lower palatability of this diet or may be a very early indicator of CKD as the literature reports that female cats had higher cadmium concentrations in the cortex of the kidneys post-mortem compared to male cats (Paßlack et al., 2014), which agreed with data from humans (Kjellstrom, 1979), however no other data collected supported a higher incidence of CKD in this group and a longer study period is required to clarify this.

Similarly fluctuations of food intake according to season have been reported both within the Massey University feline colony (Bermingham et al., 2013), and in work conducted elsewhere by others (Serisier et al., 2014). Initial feed intakes in the current study were lower than normally expected for the time of the year, although this may just indicate an adjustment period to the new diets by the cats, or a slightly lower palatability compared to their regular commercial diet. Increasing cadmium levels were not linked to reduced dietary intake as diets were consumed at similar levels across the three dietary groups. At the end of the study intakes were increasing as expected for autumn. A limitation of the study is that intake was not measured on an individual level, but rather on a group basis, meaning that a direct relationship to both weight and cadmium exposure cannot be made on an individual basis.

5.3   Blood Parameters

Chronic Kidney Disease has a complex pathogenesis; diagnostic and treatment decisions are largely based on a limited set of serological markers (such as serum creatinine), and clinical signs/symptoms (such as weight loss) (Levey et al., 2003; Weiss & Kim, 2012). There are currently no sufficiently sensitive or specific tests able to detect early kidney disease, predict disease progression, or monitor treatment response (Paepe et al., 2015). Metabolites such as creatinine (which detects kidney damage when approximately 75% of function has been lost (Von Hendy-Willson & Pressler, 2011)) and urea are the most established current kidney function markers (Paepe et al., 2015).

In the current study there were no differences in any of the CBC parameters either between groups or within groups after feeding diets ranging from 0.027 - 0.461µg Cd/g DM for 6 months (with the exception of some abnormal morphologies at baseline likely resulting from sample handling errors). It is also of note that during a routine physical examination (at the time of sampling for the CBC) cat #1706 (Toko) was diagnosed with an ear infection in week 13; however, no elevations in WBCs were captured in the CBC. While the use of a CBC can be a powerful tool to monitor response to therapy, to gauge the severity of an illness, or as a starting point for formulating a list of differential diagnoses, to gain the full benefit it must be used in conjunction with a thorough history and physical examination, a chemistry panel and urinalysis (Barger, 2003) as evidenced by Toko's ear infection.

A range of diagnostic tests were conducted to cover the minimum recommendations for diagnosing suspected feline CKD as discussed in sections 2.4 and 2.6. In addition, both approaches recommended for use in follow-up evaluations as discussed (to allow for false positive or negative results) were performed; an additional highly specific test for the condition (GFR), and follow up evaluations of the initial test (CBC and biochemistry blood panels) at a later time to detect any changes in the observed results that may increase toward or exceed the cut-off value that has high diagnostic specificity.

Urinary urea concentration is one of the routine measures used in diagnosing CKD (Finch, 2014) as discussed in Section 2.4, however urinary creatinine levels are preferred over urea as a marker of GFR (Sparkes et al., 2016). As mentioned in Section 2.1.2 amino acids excess to immediate requirements are deaminated, resulting in the toxic waste product ammonia which is further processed into urea (Elliott & Elliott, 2009). Therefore, significant differences in urea levels in the current study are likely linked to the high protein content of the diet and were not likely of clinical significance because they were within a normal range.

Creatinine (in either plasma or serum) is the most widely used surrogate marker of renal function as discussed in Section 2.4. While creatinine is an imprecise marker of GFR and lacks specificity, it is thought that if reference intervals are set low enough it may be able to detect early stage CKD (Sparkes et al., 2016). In the current study no statistical differences in serum creatinine were found either within or between the groups of cats fed the experimental diets over the 6-month trial period and all values were within the normal reference range. Overall, 29.6% of creatinine values were within the lowest quartile of the reference range (25.9 at Baseline, 33.3% at 6 Months) and 68.5% were in the upper three quartiles (74% at Baseline, 63% at 6 Months).

Increasing serum phosphate (PO_4) is also linked to the progression of CKD (Sparkes et al., 2016). While all values were within a normal range in the current study, the biochemistry panel revealed statistical differences in PO_4 levels both over time within the same group (cats fed Low and Medium cadmium diets) and between groups (cats fed the Low and High cadmium diets versus those eating the Medium cadmium diet) after 6 months. Similarly, research into dietary protein levels and the progression of experimentally induced CKD (Adams et al., 1993) found that while PO_4 values were well within the reference range, cats fed a low protein diet had a significantly lower mean PO_4 concentration than cats fed a high protein diet. Results also showed that mean serum PO_4 concentrations were not significantly different between control cats and cats with CKD fed the same diets. The authors further concluded that as the values were within the reference range they were probably not clinically significant (Adams et al., 1993). This suggests that differences in protein levels in both the experimental and their standard diets before the trial may have had some influence on PO_4 levels, although these changes were not of clinical significance and therefore unlikely to indicate changes attributable to cadmium-induced CKD.

While bone decalcification and increased urinary excretion of calcium have been associated with chronic cadmium exposure (Johri et al., 2010; Ministry for the Environment, 2011; Sigel et al., 2013), no evidence was found of calcium derangement in the biochemistry panel. This supports the idea that the adverse effects on bone and calcium metabolism may be secondary to renal damage and subsequent disruption of calcium metabolism and kinetics (Johri et al., 2010; Ministry for the Environment, 2011; Sigel et al., 2013).

5.4   Urine parameters

Changes in urinary pH have been linked to the macro-nutrient profile of a diet, with methionine and cysteine having been found to have an effect on urinary pH in cats (Jeremias et al., 2013). In feeding high protein diets to cats in the current study, the resulting higher intakes of these amino acids could have contributed to the pH values of urine being at the more acidic end of the reference range.

Mildly positive correlations have been found between GFR and USG (Paepe et al., 2015). Most cats with CKD have isothenuric urine (where USG is between 1.007–1.015) (DiBartola et al., 1987; Elliott & Barber, 1998), however isothenuria only occurs when greater than 66% of nephrons have become non-functional (Chew et al., 2011). Some cats with spontaneous or experimentally induced CKD can retain their urine concentrating ability despite being azotaemic, particularly in the early stages of disease (Ross & Finco, 1981; DiBartola et al., 1987), therefore some cats with early CKD may have a USG within the normal range.

Cats with normal renal function can have wide variations in USG (Finco, 1995; Paepe et al., 2013). Studies have found cats can have normal-to-high GFR values and poorly concentrated urine (USG <1.035) (Finco, 1995; Paepe et al., 2013; Paepe et al., 2015). Conversely, some cats with decreased GFR can have concentrated urine (USG ⩾1.035) (Paepe et al., 2015) which is in agreement with the finding that some cats with severe experimental loss of renal functional mass retained their concentrating ability (Ross & Finco, 1981).

While renal mass influences maximal urine concentrating ability, non-renal factors including intake of certain drugs (Finch, 2014), such as diuretics (often used in the treatment of acute renal failure (McClellan et al., 2006) and congestive heart failure (Rishniw & Pion, 2011)), and corticosteroids (used for the treatment of inflammatory and autoimmune diseases such as feline asthma (Viviano, 2013)), diet, or fluid intake can have a major influence on USG (Finch, 2014). Age, diet type, sex, fasting status, drinking avidity and analysis method all influence USG. While Cat #1706 (Toko) was prescribed ear drops containing a corticosteroid treatment, this was administered after the collection of the urine sample at the 3-month milestone and since treatment was only for 8 days, there was no interference with the measured USG.

Cats in the current study all had low-normal USG's. Previous work has indicated that factors that particularly increased the probability of having a USG <1.035 in apparently healthy cats included increasing moisture content of the diet and age (Rishniw & Bicalho, 2015). Because cats in the dietary groups in the current study were age matched, it is more likely that the consumption of wet diets was responsible for the low USG values.

Protein in the urine can occur in the urine due to kidney disease (Syme, 2009), diabetes (Al-Ghazlat et al., 2011) and hypertension (Syme, 2009). However no glucose was detected ruling out diabetes and there were no high BPs detected (see Section 5.6 below) ruling out hypertension. While dipsticks detected persistently elevated proteins compared to baseline readings and may indicate kidney damage, given that the most sensitive testing (see Section 5.5 below) didn't indicate kidney damage over the trial period, it is unlikely that the results of the dipstick analysis would be suggestive of CKD. In addition dipstick values below +2 cannot reliably identify less severe proteinuria due to false positive and negative results and therefore require more precise quantification by measuring urine protein: creatinine (UPC) ratios (Syme, 2009).

In the sub-staging of CKD, proteinuria is examined using a urine protein: creatinine ratio (UPC) (Sparkes et al., 2016). UPC has been found to have a mildly positive relationship with GFR (Paepe et al., 2015). A value of <0.2 is considered non-proteinuric, 0.2–0.4 borderline proteinuric, and >0.4 proteinuric (Sparkes et al., 2016). However, an increased UPC may also result from increased filtration pressure associated with higher GFR values (Paepe et al., 2015). While in the current study UPC values were not included in any of the urinalysis over the 6 month study period and could be considered a limitation, with a creatinine of <140μmol/l and without firm evidence of the presence of CKD cats, the next step in the protocol of staging CKD (International Renal Interest Society (IRIS), 2015) recommends a follow up creatinine measurement in 2-3 months at which point staging is re-evaluated according to the creatinine level.

5.5 Glomerular Filtration Rate (GFR)

GFR appears to provide the most accurate and sensitive assessment of functional renal mass and therefore of renal function. As discussed in Section 2.4.3, GFR may also be valuable for monitoring renal function following toxin exposure or recovery from acute kidney injury, although its application in these settings is yet to be fully evaluated in cats (Finch, 2014).

As discussed in Section 2.5 'normal' GFR is poorly defined in cats, and a definitive reference range has not been agreed upon (Von Hendy-Willson & Pressler, 2011), most likely due to variations in protocols (including markers used, assays for measurement of serum or urine marker concentration, urine or blood sampling times, and pharmacokinetic models used for GFR calculation), as well as the unknown effect of individual factors that may influence GFR, such as patient signalment (veterinary medical history including the animal's age, sex and breed), circadian variation, hydration status, dietary protein concentration, and the use of sedation during GFR measurement (Von Hendy-Willson & Pressler, 2011).

Iohexol plasma clearance appears to be the simplest and most accurate method for determining GFR in clinical practice (Von Hendy-Willson & Pressler, 2011) and has been validated in cats (Pressler, 2013). While the various methods of measuring iohexol clearance have produced consistent results over a variety of GFRs, there is variation between the absolute values obtained (Von Hendy-Willson & Pressler, 2011). It has also been reported that there is some day to day variability in GFR that is not related to significant biological change (van Hoek et al., 2007).

Plasma clearance of iohexol has been investigated thoroughly in cats (van Hoek et al., 2007), however analysis of the iohexol has been conducted with colorimetric assays (Miyamoto, 2001c, 2001a), atomic emission spectroscopy (Becker et al., 2000), or x-ray fluorescence (Goy-Thollot et al., 2006). One of the drawbacks of x-ray fluorescence is that it has a high detection limit, requiring both large volumes of blood (Braselton et al., 1997) and a higher dose of iohexol to be administered, thus increasing the risk of toxicity as demonstrated in humans (Barrett & Parfrey, 2006). Colorimetric assays are nonlinear above 120mg/L, which is in the range seen with renal impairment (Braselton et al., 1997). Each of these three methods effectively measures the total amount of iohexol instead of each of the two isomers (exo-iohexol and endo-iohexol). In contrast, high performance liquid chromatography (HPLC) is considered more sensitive (Braselton et al., 1997) and can measure the amount of exo-iohexol and endo-iohexol in a sample (van Hoek et al., 2007).

Analysis of iohexol concentrations in samples using HPLC allows the detection of two peaks corresponding to the two isomers of iohexol eluted with UV detection (Finch, 2014).

There is currently no agreement on a standard protocol for the measurement of the two isomers when calculating plasma clearance. Some studies have reported a difference in the clearance of the two isomers in cats (Le Garreres et al., 2007; van Hoek et al., 2007; van Hoek et al., 2008; van Hoek et al., 2009), suggesting that measuring total iohexol may lead to inaccuracies in determining GFR. In contrast, a more recent study demonstrated no significant difference in clearance between the two isomers (Finch et al., 2011) which was supported by similar findings in dogs (Laroute et al., 1999) and humans (Krutzén et al., 1984).

Pharmacokinetic models consider the body as a series of compartments a marker is distributed through, the type and number of which used in study design affects the subsequent total area under the plasma concentration-versus-time curve (AUC) (Von Hendy-Willson & Pressler, 2011). A one-compartment model views the body as a single compartment and assumes immediate distribution of the marker throughout the body. A two-compartment model assumes that clearance of a marker in plasma is due to movement from the vasculature into tissues initially, later followed by elimination from the body. In non-compartmental models, sampling time points are used to construct trapezoids defined by the curve and calculate AUC by adding the area of each trapezoid.

Several studies have attempted to determine the minimum number of blood samples required for accurate results (Von Hendy-Willson & Pressler, 2011). Recent findings, based on a non-compartmental pharmacokinetic model (which unlike one or two compartment methods is assumption free), indicate that GFR cannot be reliably estimated based on a single sample (Paepe et al., 2015). While a margin of error of <20% is considered acceptable using 3-4 samples, for research purposes it has been proposed that smaller margins of error of preferably ⩽10% and the use of five or more samples are required to estimate the GFR in most cats (Paepe et al., 2015). These results also indicated that a blood sample 10 hours after marker injection is almost always part of the optimal sampling time combination because the timing of the last sample determines the percentage of AUC extrapolated (Paepe et al., 2015). The larger this proportion, the more inaccurate the GFR estimate; the extrapolated area should never exceed 20% of the total area (Watson et al., 2002). In contrast, another study reported that at 8 hours the plasma exo-iohexol concentrations were below the level of quantitation in eight out of 12 cats studied (Le Garreres et al., 2007). More research is needed to determine a standardised approach for measuring plasma clearance of iohexol in cats.

Iohexol sampling in the current study was based on a previously published study (Heiene et al., 2009). Using a non-compartmental model lowered the margin of error in estimating GFR. Based on more recent research while the use of four samples as in the current study provides acceptable results, (Paepe et al., 2015), the addition of a further fifth sample may have been required for further lower margins of error.

Fluctuations in GFR were observed both within and between dietary groups over the Baseline, 3 and 6 Month milestones of the current study, although most importantly there were no significant differences between groups at the conclusion of the study. A number of non-renal factors may be responsible for this including age and diet as discussed below.

Age is thought to play a part in differing GFR values with increasing age though to have a degenerative effect on the kidney and therefore decreasing glomerular function and GFR, although this area is not well researched in cats (van Hoek et al., 2007). While the groups of cats in the current study were age-matched, there is some spread in the ages of individual cats with the youngest being approximately 3 years old and the oldest 9 years old. While no significant age effect for clearance of exo- or endo-iohexol was found in one study (van Hoek et al., 2007), the same study found a significant difference in creatinine clearance between young (age 7–12 months) and aged cats (age 9–12 years). Conversely another study using both iohexol and creatinine in 57 cats with a wider age range (1–17 years) indicated that GFR was not affected by age (Heiene et al., 2009). No evidence was found in the current study to suggest any age differences in GFR independent of treatment group. Further studies are needed to elucidate the effect of age on GFR.

It is possible that the restricted access to food (and its high moisture content) during iohexol sampling induced a level of mild dehydration, although no evidence for this was observed. Some degree of dehydration is thought to be associated with a reduced GFR due to a reduced extracellular fluid volume (Reynolds et al., 2014), and has been reported in dogs (Tabaru et al., 1993).

As discussed in section 2.4, direct measures of GFR are considered more accurate than the use of biomarkers; however the use of techniques such as iohexol clearance is often not practicable in general practice (Paepe & Daminet, 2013; Quimby, 2015). While biomarkers may have allowed the detection of CKD following the dietary exposure to cadmium, additional work in this area is still needed and many fundamental issues regarding the selection of markers and definition of their critical levels have yet to be fully resolved (Prozialeck & Edwards, 2010).

5.6 Blood Pressure

Chronic Kidney Disease and hypertension are both common in older cats. While the current consensus is that hypertension is secondary to CKD, the possibility still exists that hypertension occurs independently of the presence of CKD or is actually a cause of renal injury (Syme et al., 2002; Brown et al., 2007; Bijsmans et al., 2015; Paepe et al., 2015).

Hypertension can be associated with organ damage and should be addressed promptly, making measurement of blood pressure (BP) critical in sick and at risk animals (Jepson, 2011; Stepien, 2011). The measurement of BP is also an important part of the clinical staging of CKD (Quimby et al., 2011). While no long term change in BP was measured over the 6 month period of the current study and thus could be considered a limitation, examining the mean group values of systolic blood pressure (SBP) (i.e. cats fed the Low, Medium, or High cadmium diets) at the end of the study sub-staged them as normotensive according to CKD guidelines (Sparkes et al., 2016).

The diagnosis and management of systemic hypertension in cats requires a reliable method for measurement of systemic arterial BP. Unfortunately, the act of measuring BP and the setting in which the measurement is taken, both raise BP and heart rate (HR) in cats, an effect referred to as the white-coat effect (Belew et al., 1999).

Following simulated vet clinic visits, the SBP measured by radiotelemetry in healthy cats during the examination exceeded the 24-hour average by 17.6mmHg with considerable inter-cat variability and with SBP rates of between 75.3 - 227.2mmHg (Belew et al., 1999). In cats with renal disease the mean increase in SBP during the examination (22.3 ±0.9 mmHg) was significantly greater than in the healthy cats with the magnitude of the white-coat effect tending to decrease rather than disappear over the duration of the visit. Similarly the magnitude of the white-coat effect varied when cats were subjected to 5 repeat visits, but did not reduce in the group as a whole (Belew et al., 1999). Comparatively, another study mimicked the conditions of a clinical setting and measured SBP in 7 healthy cats (mean age 5.9 years) 7 times over a 10-day period with ≥24 hours between measurements. Again mean SBP varied by as much as 47mmHg between cats and as much as 36 mmHg within a cat (Sparkes et al., 1999).

In the current study, mean group measurements (i.e. cats fed the Low, Medium, or High cadmium diets) varied by a maximum of 25mmHg across groups at the end of the study and 23mmHg within a group (Low cadmium diet). Maximal HR was 42 BPM across groups and 31 BPM within a group (High cadmium diet). This is lower than the variability recorded by Belew (1999) and Sparkes (1999), and may reflect the higher levels of socialisation and handling in the colony animals.

Statistically significant differences have been documented between the home environment and the clinic environment for both blood pressure and heart rate. The highest documented increase on an individual basis in one study examining differences between the home and clinic was 31mmHg, and the largest increase in HR was 76 BPM (Quimby et al., 2011). These changes are large enough to be considered clinically relevant; for example, in the staging of CKD, a difference in BP of >10mmHg is enough to move a cat from the 'low risk' to 'moderate risk', or 'moderate risk' to 'high risk' substage (Quimby et al., 2011; Sparkes et al., 2016). Accordingly, SBP values ≥180mmHg are unlikely to reflect white-coat hypertension and should be treated as clinically relevant when paired with a full diagnostic evaluation for organ damage and underlying disease. In a cat with known CKD, an SBP >160mmHg should be considered clinically relevant (Stepien, 2011).

Because the cats in the current study were not removed from their familiar surroundings for blood pressure and heart rate assessment and are used to being handled, their measurements may be more akin to that of a home environment. None of the mean group SBP values were above either threshold value (180 or 160mmHg), and when paired with the other diagnostic parameters (such as blood and urine) there is no evidence of organ damage, suggesting there is no evidence of hypertension and may support the theory that hypertension is secondary to CKD. Overall there was no suggestion that the differing levels of cadmium in the diet had a measurable effect on blood pressure in the cats.

5.7   Conclusion

This study aimed to examine the effect of feeding variable levels of cadmium in cat food on renal function in cats. While cadmium levels in some cat foods (particularly wet diets) are higher than safe recommended levels for human consumption, the level of risk is specific to where the ingredients for the cat food are sourced from. The results of the current study indicated that the current dietary cadmium levels appear to be too low to have a biological effect in cats, and indicate that no change needs to be made to current practice in using offal diverted from human consumption in cat foods.

More work is needed to explore the potential for genetic and/or functional differences in mechanisms which are involved in the transport, and/or deposition of cadmium, or are protective against cadmium toxicity in cats. While the current study was unable to contribute to the mechanistic process of cadmium mediated CKD, it is also important to use a whole animal model in the target species to fully explore complex biological interactions.

A trend of weight loss over the study was observed in females (who may be more susceptible than males to cadmium deposition and therefore CKD), particularly those on the High cadmium diet. A longer study period is required to further clarify if this was a result of natural biological variation or a very early sign of CKD attributable to cadmium toxicity. These animals will continue to be monitored for signs of CKD given this dietary intervention.

Pairing blood and urine parameters, which are used as diagnostic tools for CKD, showed no consistent statistical differences between the measurements at each milestone of the study. However, none of these values on a group basis were outside of a normal reference range and appear to be explained by other external factors such as diet.

This is the first time that GFR has been used to explore cadmium mediated CKD in cats. Overall there was no difference in GFR between the start and end of the study which provides a clear indication that the levels of cadmium currently in offal used in the making of the cat food in New Zealand do not affect kidney function when fed for 6 months. This research extends knowledge of GFR in cats and serves as a basis for future studies to further define normal parameters and standard approaches in measuring GFR in cats.

Overall given the lack of effect of cadmium across the study groups it is unlikely to be a significant contributor to CKD, although there remains the possibility that longer-term consumption may contribute to the disease. An ideal future longer (year-long) study would perhaps use older groups of cats (e.g. 8 years old), who are likely to develop CKD in the near future, or groups of cats with or without CKD. The addition of analysis of cadmium levels in urine in future studies would also contributed to picture of what proportion is being excreted versus building up in the kidneys

Appendices

Appendix 1.1   Experimental Group Composition

Study ID	Cat Name	Diet	Age at 16/10/14	Gender
1601	Fox	Low	2 years, 9 months	Male
1602	Ali	Low	6 years, 1 month	Male
1603	Rota	Low	9 years, 0 months	Female
1604	Olly	Low	7 years, 6 months	Male
1605	Meg	Low	8 years, 10 months	Female
1606	Gunna	Low	8 years, 8 months	Male
1607	Nonu	Low	6 years, 1 months	Male
1608	Caro	Low	8 years, 11 months	Female
1609	Red	Low	2 years, 9 months	Male
1801	Yale	Medium	7 years, 9 months	Male
1802	Nemo	Medium	2 years, 9 months	Male
1803	Pepsi	Medium	8 years, 8 months	Female
1804	Davey	Medium	4 years, 8 months	Male
1805	Badal	Medium	7 years, 9 months	Male
1806	Zaba	Medium	8 years, 10 months	Female
1807	Sione	Medium	6 years, 7 months	Male
1808	Pandy	Medium	7 years, 8 months	Female
1809	Molly	Medium	7 years, 8 months	Female
1703	Mini	High	8 years, 7 months	Female
1704	Nico	High	5 years, 0 months	Male
1705	Beau	High	7 years, 6 months	Male
1706	Toko	High	8 years, 8 months	Female
1708	Richi	High	7 years, 4 months	Male
1707	Thys	High	7 years, 9 months	Male
1709	Jaz	High	8 years, 7 months	Female

Appendix 1.2   Detailed Experimental Timeline

Date	Day	Week	Month	Event
10/10/2013	-8	-1	-1	All Pens: CBC
14/10/2013	-4	0	0	Pen 16: Urinalysis, Iohexol, Weighed
15/10/2013	-3	0	0	Pen 17: Urinalysis, Iohexol, Weighed
16/10/2013	-2	0	0	All Pens: Weighed
17/10/2013	-1	0	0	Pen 18: Urinalysis, Iohexol, Weighed
18/10/2013	1	0	0	All Pens: Diets started
23/10/2013	6	1	0	All Pens: Weighed
30/10/2013	13	2	0	All Pens: Weighed
06/11/2013	20	3	0	All Pens: Weighed
13/11/2013	27	4	1	All Pens: Weighed
14/11/2013	28	4	1	All Pens: CBC, Urinalysis
20/11/2013	34	5	1	All Pens: Weighed
27/11/2013	41	6	1	All Pens: Weighed
04/12/2013	48	7	1	All Pens: Weighed
11/12/2013	55	8	2	All Pens: Weighed
12/12/2013	56	8	2	All Pens: CBC, Urinalysis
18/12/2013	62	9	2	All Pens: Weighed
25/12/2013	69	10	2	All Pens: Weighed
01/01/2014	76	11	2	All Pens: Weighed
08/01/2014	83	12	3	All Pens: Weighed
13/01/2014	88	13	3	Pen 16: Urinalysis, Iohexol, Weighed
14/01/2014	89	13	3	Pen 17: Urinalysis, Iohexol, Weighed
15/01/2014	90	13	3	All Pens: Weighed
16/01/2014	91	13	3	All Pens: CBC, Urinalysis Pen 18: (in addition) Iohexol, Weighed
22/01/2014	97	14	3	All Pens: Weighed
29/01/2014	104	15	3	All Pens: Weighed
05/02/2014	111	16	4	All Pens: Weighed
12/02/2014	118	17	4	All Pens: Weighed
13/02/2014	119	17	4	All Pens: CBC, Urinalysis
19/02/2014	125	18	4	All Pens: Weighed
26/02/2014	132	19	4	All Pens: Weighed
05/03/2014	139	20	5	All Pens: Weighed
12/03/2014	146	21	5	All Pens: Weighed
13/03/2014	147	21	5	All Pens: CBC, Urinalysis
19/03/2014	153	22	5	All Pens: Weighed
26/03/2014	160	23	5	All Pens: Weighed
02/04/2014	167	24	6	All Pens: Weighed, Blood Pressure
09/04/2014	174	25	6	All Pens: Weighed, Blood Pressure
10/04/2014	175	25	6	All Pens: CBC, Urinalysis
14/04/2014	179	26	6	Pen 16: Urinalysis, Iohexol, Weighed
15/04/2014	180	26	6	Pen 17: Urinalysis, Iohexol, Weighed
16/04/2014	181	26	6	All Pens: Weighed, Blood Pressure
17/04/2014	182	26	6	Pen 18: Urinalysis, Iohexol, Weighed

Appendix 1.2   Detailed Experimental Timeline

Appendix 1.3   Iohexol sampling times and corresponding iodine concentrations

Low Diet Group

Animal	Baseline		3 Months		6 Months	
	Time Elapsed (Hours: Minutes)	Iodine Concentration (µg/mL)	Time Elapsed (Hours: Minutes)	Iodine Concentration (µg/mL)	Time Elapsed (Hours: Minutes)	Iodine Concentration (µg/mL)
Fox	1:45	147.57	1:33	268.33	1:41	112.54
	3:01	81.36	2:45	56.29	2:54	34.95
	4:01	39.07	3:47	31.97	3:56	16.27
	5:15	33.69	5:00	18.21	5:11	8.12
Ali	1:46	163.89	1:39	130.34	1:48	144.99
	3:03	61.74	2:54	49.46	3:03	108.87
	4:03	36.39	3:53	28.09	4:03	57.38
	5:16	14.37	5:09	12.92	5:20	30.14
Rota	1:49	127.10	1:46	128.34	2:02	40.14
	3:05	130.01	3:05	49.66	3:16	8.66
	4:04	63.59	3:56	29.17	4:17	5.55
	5:23	22.98	5:17	17.42	5:32	2.16
Olly	1:47	100.35	1:59	148.70	2:16	80.23
	3:02	43.28	3:15	76.44	3:34	36.48
	4:03	22.38	4:14	49.55	4:31	33.38
	5:17	11.17	5:30	26.52	5:49	13.85
Meg	1:48	89.55	2:06	150.34	2:27	67.15
	3:03	33.46	3:24	81.69	3:44	26.54
	4:03	18.11	4:23	59.58	4:43	12.25
	5:16	7.62	5:38	27.84	5:56	6.57
Gunna	1:51	98.37	2:14	94.45	2:34	50.26
	3:03	43.03	3:28	44.71	3:51	18.58
	4:05	24.69	4:29	30.63	4:47	8.75
	5:18	12.07	5:43	13.87	6:02	5.46
Nonu	1:50	325.26	2:23	141.59	2:42	40.56
	3:02	88.99	3:38	63.31	3:57	17.14
	4:01	42.36	4:37	37.37	5:01	4.74
	5:21	18.59	5:54	18.63	6:13	2.26
Caro	1:48	51.74	2:34	105.53	1:36	154.20
	3:03	24.66	3:47	51.76	2:50	50.49
	4:03	15.48	4:50	25.99	3:56	20.79
	5:16	7.24	6:11	13.42	5:05	8.21
Red	1:46	146.95	1:49	115.42	2:09	62.52
	3:02	64.46	3:05	51.87	3:25	28.44
	4:01	34.41	4:04	21.86	4:24	13.14
	5:16	16.93	5:20	11.36	5:41	7.98

Medium Diet Group

Animal	Baseline		3 Months		6 Months	
	Time Elapsed (Hours: Minutes)	Iodine Concentration (μg/mL)	Time Elapsed (Hours: Minutes)	Iodine Concentration (μg/mL)	Time Elapsed (Hours: Minutes)	Iodine Concentration (μg/mL)
Yale	1:45	193.52	1:43	171.10	2:39	89.64
	2:58	100.09	2:59	71.36	3:54	45.26
	3:59	59.83	3:56	40.05	4:47	28.13
	5:15	34.55	5:12	18.98	6:02	14.58
Nemo	1:52	159.72	1:51	148.14	1:40	171.52
	3:11	80.77	3:06	68.21	2:57	81.19
	4:08	47.57	4:07	26.57	4:00	42.49
	5:29	19.84	5:25	13.60	5:12	22.60
Pepsi*	1:45	162.17	2:01	47.92	1:56	74.44
	3:01	73.99	3:16	13.41	3:17	24.79
	4:00	42.36	4:15	4.98	4:18	10.28
	5:17	20.28	5:30	2.50	5:30	5.00
Davey	1:45	166.57	2:07	72.17	1:56	163.59
	3:02	83.83	3:23	30.20	3:17	79.62
	4:02	50.60	4:23	15.56	4:11	40.20
	5:15	23.59	5:39	7.76	5:26	19.59
Badal	1:47	138.68	2:15	88.44	1:32	206.04
	3:02	69.24	3:29	33.40	2:48	113.51
	4:02	38.81	4:31	11.75	3:47	60.41
	5:16	21.13	5:43	6.90	5:03	33.37
Zaba	1:44	230.35	2:24	63.11	2:11	51.38
	3:00	95.30	3:31	12.35	3:30	13.96
	4:01	47.47	4:38	5.24	4:29	5.35
	5:16	24.73	5:55	1.71	5:34	2.59
Sione	1:50	93.08	2:35	37.44	2:31	44.13
	3:06	40.08	3:50	14.26	3:44	13.75
	4:13	18.55	4:49	8.64	4:51	5.02
	5:15	9.23	6:05	4.37	5:59	3.54
Pandy	1:45	162.00	2:42	44.19	2:20	101.90
	3:01	83.51	3:57	15.84	3:35	47.31
	4:02	41.97	4:59	7.64	4:37	25.16
	5:17	20.60	6:13	3.92	5:49	12.49
Molly	1:44	156.20	2:54	35.85	1:49	89.29
	3:00	73.39	4:10	14.21	3:08	39.89
	3:59	33.64	5:07	7.14	4:06	18.88
	5:18	12.74	6:22	4.08	5:19	9.24

*Pepsi - fractious during injection, Iohexol administered may be less than stated dose

High Diet Group

Animal	Baseline		3 Months		6 Months	
	Time Elapsed (Hours: Minutes)	Iodine Concentration (µg/mL)	Time Elapsed (Hours: Minutes)	Iodine Concentration (µg/mL)	Time Elapsed (Hours: Minutes)	Iodine Concentration (µg/mL)
Muse	1:47	87.07	1:47	49.56	1:37	82.93
	3:06	33.16	3:04	16.06	2:51	35.56
	4:04	20.01	4:02	8.17	3:51	12.95
	5:21	8.70	5:16	3.73	5:05	6.19
Carta	1:43	138.30	1:54	151.92	1:44	109.92
	2:59	53.15	3:11	76.24	2:59	51.50
	3:59	32.77	4:11	46.30	3:58	27.63
	5:19	17.60	5:27	26.11	5:16	15.31
Mini	1:45	134.40	2:03	67.66	1:53	68.88
	3:01	51.11	3:19	33.87	3:11	22.63
	4:02	26.85	4:21	17.22	4:10	10.94
	5:27	12.16	5:34	9.19	5:25	5.73
Nico	1:45	80.70	2:07	62.67	1:57	74.95
	3:01	28.56	3:21	30.86	3:15	32.01
	4:01	13.82	4:25	15.07	4:14	13.80
	5:18	4.27	5:40	6.74	5:31	5.87
Beau	1:45	109.04	2:19	70.96	2:04	109.61
	3:02	50.58	3:31	25.44	3:19	54.35
	4:02	31.73	4:35	9.48	4:21	36.52
	5:17	14.91	5:51	8.64	5:37	18.05
Toko	1:47	120.06	2:36	45.32	2:16	57.07
	3:05	49.64	4:45	8.41	3:33	23.56
	4:07	25.71	*	*	4:32	12.78
	5:17	12.11	5:54	4.55	5:47	5.62
Thys	1:48	229.94	2:31	120.40	2:26	130.67
	3:01	115.58	3:47	66.25	3:41	75.24
	4:04	56.91	4:49	29.38	4:43	48.53
	5:16	36.00	6:03	22.80	5:56	28.55
Richi	1:48	93.23	2:41	32.81	2:39	85.72
	3:03	44.16	3:56	12.66	3:52	46.69
	4:04	21.34	4:59	5.26	4:51	28.06
	5:17	7.82	6:11	2.23	6:05	15.57
Jaz	1:53	108.28	2:49	25.20	2:47	38.50
	3:07	46.62	4:06	10.24	4:00	13.67
	4:05	23.59	5:08	5.28	5:06	6.20
	5:24	10.71	6:22	2.38	6:16	3.47

*No "3h" sample

Appendix 1.4   Raw food intake data

Mean Weekly food intake on a group basis (g)

Week	Diet		
	Low	Medium	High
0	2174.00	2061.67	2394.33
1	2175.86	2038.43	2392.86
2	2101.71	2018.71	2487.00
3	2059.57	2031.71	2430.43
4	2316.43	2020.00	2471.00
5	2046.14	1887.14	2439.71
6	2326.57	2146.43	2698.00
7	2388.00	2145.57	2580.00
8	2315.86	2120.57	2829.71
9	2407.57	2109.00	2865.57
10	2517.57	2193.57	2969.86
11	2557.57	2257.57	2972.43
12	2572.71	2186.86	2828.29
13	2329.86	2083.86	3003.71
14	2332.43	2220.57	2936.71
15	2316.71	1987.14	2980.71
16	2406.43	2126.86	2770.29
17	2450.14	2034.14	2903.14
18	2411.71	2033.14	3068.57
19	2317.29	2101.86	3049.86
20	2411.43	2200.43	3184.86
21	2335.57	2243.14	3005.14
22	2452.43	2148.29	3264.29
23	2595.57	2216.57	3151.00
24	2485.57	2152.00	3207.86
25	2452.57	2054.43	2971.71
26	2799.00	2323.00	3240.33

Appendix 1.5   Individual Weekly Weights

Low Diet Group (g)

Animal	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Red	3922	3899	3862	3920	3885	3899	3931	3901	3888
Nonu	4258	4153	4115	4036	4061	4044	4072	4018	4033
Gunna	4929	4798	4663	4641	4690	4617	4520	4446	4530
Caro	3036	2916	3089	2888	2906	2983	2997	2976	2951
Olly	3340	3269	3229	3209	3254	3166	3150	3151	3185
Ali	4134	4120	4180	4143	4104	4089	4179	4178	4216
Fox	3744	3749	3682	3689	3674	3725	3723	3620	3781
Rota	3369	3227	3079	2890	2836	2904	2853	2922	2988
Meg	4086	3994	3956	3957	3958	3901	3973	3936	3933
MEAN	**3869**	**3792**	**3762**	**3708**	**3708**	**3703**	**3711**	**3683**	**3723**

Animal	Week 9	Week 10	Week 11	Week 12	Week 13	Week 14	Week 15	Week 16	Week 17
Red	3990	3898	3992	4056	3995	3967	3968	3978	4011
Nonu	4050	4080	4088	4146	4102	4083	4023	4006	4011
Gunna	4544	4498	4538	4565	4623	4695	4509	4567	4635
Caro	3021	2977	3119	2988	2982	2903	2879	2891	2928
Olly	3155	3128	3190	3210	3201	3181	3165	3166	3178
Ali	4243	4261	4377	4305	4417	4241	4309	4327	4330
Fox	3813	3816	3801	3812	3863	3751	3815	3806	3892
Rota	2867	2988	3044	3147	3175	3019	3037	2989	3023
Meg	4003	3932	4020	4025	4009	4004	4005	3956	4004
MEAN	**3743**	**3731**	**3797**	**3806**	**3819**	**3760**	**3746**	**3743**	3779

Animal	Week 18	Week 19	Week 20	Week 21	Week 22	Week 23	Week 24	Week 25	Week 26
Red	3998	3970	3970	4038	4064	4119	4179	4116	4180
Nonu	3917	4001	3945	3908	3938	3979	4019	4097	4124
Gunna	3550	4510	4618	4555	4642	4633	4663	4587	4763
Caro	2946	2979	2914	2959	2995	2981	3036	2999	3162
Olly	3188	3171	3173	3152	3094	3194	3106	2934	3032
Ali	4270	4235	4231	4265	4287	4227	4273	4279	4144
Fox	3895	3922	3893	3927	4003	4089	4085	4104	4161
Rota	2974	2916	2947	2840	2948	3080	3143	3190	3224
Meg	4092	4051	4087	4103	4094	3989	4078	4059	3961
MEAN	**3648**	**3751**	**3753**	**3750**	**3785**	**3810**	**3842**	**3818**	**3861**

Individual Weekly Weights (g): Medium Diet Group

Animal	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Yale	4640	4659	4395	4431	4261	4262	4235	4168	4202
Davey	4290	4159	4072	4071	3906	3952	3880	3876	3772
Nemo	3291	3431	3263	3472	3329	3491	*	3465	3551
Sione	3263	3187	3162	3197	3086	3140	3182	3304	3157
Zaba	3245	3132	3024	3033	2952	3044	2999	2971	2950
Pepsi	3630	3643	3439	3528	3408	3464	3399	3441	3420
Pandy	4103	3855	3763	3606	3571	3516	3586	3615	3517
Molly	3701	3726	3705	3643	3715	3667	3638	3676	3634
Badal	5423	5455	5254	5325	5181	5314	5385	5230	5411
MEAN	**3954**	**3916**	**3786**	**3812**	**3712**	**3761**	**3788**	**3750**	**3735**

* Not measured

Animal	Week 9	Week 10	Week 11	Week 12	Week 13	Week 14	Week 15	Week 16	Week 17
Yale	4110	4116	4221	4248	4113	4279	4320	4263	4250
Davey	3692	3643	3717	3785	3870	3856	3793	3763	3765
Nemo	3498	3545	3702	3759	3675	2624	3781	3663	3680
Sione	3240	3211	3214	3253	3295	3314	3266	3264	3277
Zaba	2930	2851	2968	3051	3018	2964	3030	2919	3058
Pepsi	3327	3324	3410	3482	3471	3415	3507	3470	3524
Pandy	3518	3624	3641	3739	3728	3688	3643	3669	3713
Molly	3605	3590	3625	3609	3656	3686	3655	3638	3540
Badal	5150	5192	5410	5341	5268	5137	5309	5346	5220
MEAN	**3674**	**3677**	**3768**	**3807**	**3788**	**3663**	**3812**	**3777**	**3781**

Animal	Week 18	Week 19	Week 20	Week 21	Week 22	Week 23	Week 24	Week 25	Week 26
Yale	4264	4241	4249	4349	4249	4257	4377	4230	4273
Davey	3812	3793	3869	3928	3929	3964	4081	4105	4148
Nemo	3752	3858	3813	3774	3863	3954	3985	4161	3946
Sione	3264	3235	3200	3168	3179	3202	3268	3070	3059
Zaba	3072	2912	3032	3064	3007	3073	2999	2685	2783
Pepsi	3533	3559	3546	3577	3591	3392	3585	3475	3476
Pandy	3639	3719	3718	3650	3800	3720	3738	3672	3656
Molly	3541	3521	3442	3405	3449	3412	3432	3391	3410
Badal	5239	5201	5053	5293	5307	5169	5312	5177	5182
MEAN	**3791**	**3782**	**3769**	**3801**	**3819**	**3794**	**3864**	**3774**	**3770**

Individual Weekly Weights (g): High Diet Group

Animal	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Toko	3528	3552	3443	3440	3358	3354	3431	3411	3491
Mini	3296	3136	3113	3179	3117	3089	3112	3100	3169
Beau	3951	3903	3813	3779	3846	3823	3916	3791	3842
Nico	3856	3829	3768	3755	3716	3664	3689	3719	3725
Jaz	4159	4041	3997	3871	3945	3881	3898	3912	3849
Thys	4729	4579	4552	4529	4512	4482	4548	4424	4648
Muse	3124	3191	3089	3142	3199	3168	3125	3055	3162
Carta	3865	3837	3722	3808	3942	3791	3784	3741	3795
Richie	3921	3835	3739	3740	3737	3749	3968	3820	3787
MEAN	**3825**	**3767**	**3693**	**3694**	**3708**	**3667**	**3719**	**3664**	**3719**

Animal	Week 9	Week 10	Week 11	Week 12	Week 13	Week 14	Week 15	Week 16	Week 17
Toko	3449	3382	3381	3291	3188	3437	3385	3385	3398
Mini	3149	3122	3102	3158	3159	3151	3138	3127	3066
Beau	3754	3781	3923	3869	3922	3786	3761	3900	3739
Nico	3663	3697	3757	3705	3841	3733	3837	3883	3839
Jaz	3839	3800	3858	3825	3916	3879	3746	3784	3739
Thys	4371	4384	4504	4393	4413	4394	4426	4357	4341
Muse	3157	3187	3279	3291	3269	3274	3295	3318	3380
Carta	3809	3832	3804	3872	3915	3888	3889	3925	3919
Richie	3855	3787	3865	3708	3812	3717	3676	3713	3769
MEAN	**3672**	**3664**	**3719**	**3679**	**3715**	**3695**	**3684**	**3710**	**3688**

Animal	Week 18	Week 19	Week 20	Week 21	Week 22	Week 23	Week 24	Week 25	Week 26
Toko	3207	3159	3159	3166	3166	3070	3117	2961	3006
Mini	3094	3088	3120	3114	3179	3170	3070	3171	3206
Beau	3729	3802	3849	3772	3802	3895	3869	3848	3873
Nico	3906	3872	3982	3945	4031	4028	4119	4016	4127
Jaz	3788	3746	3727	3716	3700	3689	3810	3706	3736
Thys	4357	4331	4323	4243	4429	4452	4490	4292	4362
Muse	3307	3363	3422	3404	3562	3508	3589	3673	3723
Carta	3981	3927	3954	3903	3971	3943	4006	3987	3981
Richie	3743	3726	3757	3670	3760	3849	3882	3828	3821
MEAN	**3679**	**3668**	**3699**	**3659**	**3733**	**3734**	**3772**	**3720**	**3759**

Appendix 1.6   Individual CBC results

Key: ▊ Below Reference Value   ▊ Above Reference Value

Low Diet Group, Month 0

Cat	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLAT	SEGN	LYMPH	MONO	EOSIN	BASO	BAND
Fox	12.3	8.1	118	0.32	39	15	372	Poikilocytosis + Keratocytes	6.15	4.92	0.62	0.62	0.17	0.00
Ali	7.2	8.3	123	0.36	42	15	346	Poikilocytosis + Keratocytes	4.03	2.74	0.14	0.29	0.00	0.00
Rota	12.7	8.0	119	0.35	44	15	339	Poikilocytosis + Howell-Jolly bodies + Heinz bodies	6.10	3.94	0.13	1.65	0.89	0.00
Olly	10.2	8.0	117	0.32	40	15	366	Poikilocytosis Keratocytes + Rouleaux	4.69	4.49	0.20	0.61	0.20	0.00
Meg	10.4	9.2	115	0.33	36	13	354	Poikilocytosis + Keratocytes	5.41	3.95	0.00	0.94	0.10	0.00
Gunna	16.7	8.9	125	0.35	40	14	357	Red cells appear normal	9.19	5.85	0.84	0.67	0.17	0.00
Nonu	9.6	8.2	118	0.37	46	14	316	Rouleaux	7.10	1.82	0.29	0.38	0.00	0.00
Caro	12.1	8.6	125	0.40	46	15	314	Rouleaux + Platelets clumped. White cells show Reactive lymphocytes		7.74	3.87	0.12	0.36	0.00
Red	8.3	8.5	119	0.34	40	14	355	Poikilocytosis + Keratocytes + Rouleaux	4.15	2.41	0.75	1.00	0.00	0.00

Individual CBC results – Medium Diet Group, Month 0

Cat	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLAT	SEGN	LYMPH	MONO	EOSIN	BASO	BAND
Yale	17.0	9.9	143	0.41	41	14	247	Poikilocytosis + Kerocytes + Rouleaux	12.41	3.74	0.17	0.51	0.17	0.00
Nemo	14.0	9.1	138	0.39	42	15	357	Red cells appear normal	11.20	1.96	0.28	0.56	0.00	0.00
Pepsi	17.7	8.4	122	0.35	41	15	351	Red cells appear normal	13.28	3.01	0.18	1.24	0.00	0.00
Davey	14.6	9.7	148	0.43	44	15	343	Red cells appear normal	7.45	6.57	0.15	0.44	0.00	0.00
Badal	8.1	8.2	107	0.30	37	13	354	Rouleaux	4.37	3.08	0.16	0.41	0.08	0.00
Zaba	11.5	8.7	123	0.34	39	14	361	Poikiloyctosis + Keratocytes	7.25	3.22	0.58	0.35	0.12	0.00
Sione	13.0	8.9	129	0.35	39	15	374	Poikilocytosis +Keratocytes	9.75	2.21	0.26	0.78	0.00	0.00
Pandy	11.7	8.4	123	0.33	40	15	368	Poikilocytosis + Kerocytes	7.37	3.51	0.12	0.70	0.00	0.00
Molly	14.3	8.9	117	0.33	37	13	353	Poikilocytosos + Keratocytes, White cells show reactive lymphocytes occ.	10.30	2.57	0.57	0.86	0.00	

Individual CBC results – High Diet Group, Month 0

Cat	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLAT	SEGN	LYMPH	MONO	EOSIN	BASO	BAND
Muse	24.0	8.9	129	0.39	44	15	331	Poikilocytosis + Keratocytes + Howell-Jolly bodies occ. Rouleaux	18.84	5.52	0.96	1.68	0.00	0.00
Carta	10.6	9.4	133	0.38	40	14	355	Poikilocytosis + Keratocytes	5.72	3.39	0.21	0.95	0.32	0.00
Mini	10.4	7.9	116	0.32	41	15	359	Poikilocytosis + Keratocytes	4.37	4.78	0.42	0.83	0.00	0.00
Nico	13.9	10.3	151	0.43	42	15	352	Poikilocytosis + Keratocytes	7.37	5.84	0.28	0.28	0.14	0.00
Beau	8.0	8.7	121	0.33	38	14	363	Rouleaux	4.40	2.96	0.24	0.32	0.08	0.00
Toko	9.4	8.8	124	0.36	41	14	343	Red cells appear normal	7.52	1.13	0.19	0.56	0.00	0.00
Thys	11.2	9.5	123	0.36	38	13	353	Rouleaux	6.27	3.92	0.22	0.78	0.00	0.00
Richi	9.7	9.8	131	0.38	39	13	349	Poikilocytosis + Keratocytes occ. + Rouleaux	4.66	4.46	0.19	0.39	0.00	0.00
Jaz	9.3	10.8	147	0.44	41	14	332	Red cells appear normal, white cells show reactive lymphocytes	5.21	3.44	0.19	0.47	0.00	0.00

Individual CBC results – Low Diet Group, Month 1

Cat	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLAT	SEGN	LYMPH	MONO	EOSIN	BASO	BAND
Fox	13.5	9.2	130	0.36	39	14	361	485	8.10	3.80	0.70	0.70	0.30	0.00
Ali	10.5	8.6	120	0.37	43	14	324	n/a	8.40	1.70	0.10	0.30	0.00	0.00
Rota	14.0	8.6	120	0.35	41	14	345	n/a	7.10	4.60	0.10	1.40	0.70	0.00
Olly	12.9	8.2	115	0.33	40	14	354	n/a	7.40	4.60	0.10	0.60	0.10	0.00
Meg	13.4	8.8	111	0.31	35	13	360	624	8.80	2.80	0.30	1.30	0.10	0.00
Gunna	15.9	9.9	141	0.39	40	14	359	837	6.70	7.20	0.60	0.80	0.20	0.00
Nonu	No sample													
Caro	13.7	9.4	133	0.44	46	14	305	500	8.40	3.80	0.50	1.00	0.00	0.00
Red	7.8	8.7	122	0.34	40	14	355	427	4.30	2.70	0.50	0.30	0.00	0.00

Individual CBC results – Medium Diet Group, Month 1

Cat	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLAT	SEGN	LYMPH	MONO	EOSIN	BASO	BAND
Yale	15.8	9.8	136	0.40	40	14	344	619	9.60	4.90	0.50	0.80	0.00	0.00
Nemo	11.8	9.1	139	0.39	43	15	353	n/a	9.00	1.90	0.40	0.20	0.00	0.00
Pepsi	20.5	8.8	124	0.35	40	14	352	552	12.50	7.00	0.20	0.80	0.00	0.00
Davey	13.8	9.7	145	0.42	43	15	349	393	7.87	4.55	0.41	0.97	0.00	0.00
Badal	10.6	7.9	101	0.29	37	13	352	n/a	8.10	1.80	0.30	0.30	0.00	0.00
Zaba	12.1	8.5	121	0.34	40	14	360	666	9.00	1.90	0.40	0.50	0.00	0.00
Sione	12.4	8.9	129	0.34	39	15	376	577	6.30	5.70	0.10	0.20	0.00	0.00
Pandy	12.1	9.5	129	0.37	39	14	350	657	8.70	2.20	0.50	0.40	0.00	0.00
Molly	21.6	8.1	108	0.31	38	13	352	n/a	13.80	4.80	0.40	2.60	0.00	0.00

Individual CBC results – High Diet Group, Month 1

Cat	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLAT	SEGN	LYMPH	MONO	EOSIN	BASO	BAND
Muse	19.1	10.8	162	0.51	47	15	320	n/a	17.00	1.50	0.00	0.40	0.20	0.00
Carta	11.3	8.6	123	0.34	40	14	361	n/a	7.30	2.30	0.30	1.40	0.00	0.00
Mini	9.6	7.6	107	0.30	40	14	352	463	5.80	3.50	0.10	0.30	0.00	0.00
Nico	14.9	8.5	125	0.36	43	15	343	n/a	11.00	3.00	0.10	0.30	0.40	0.00
Beau	9.4	8.4	114	0.32	38	14	357	n/a	6.40	2.30	0.40	0.40	0.00	0.00
Toko	9.6	8.9	122	0.37	42	14	330	608	6.80	1.40	0.20	1.10	0.10	0.00
Thys	11.2	9.5	126	0.36	37	13	354	n/a	7.50	2.80	0.20	0.60	0.10	0.00
Richi	10.8	9.1	121	0.35	39	13	348	489	6.48	2.92	0.11	0.54	0.43	0.00
Jaz	12.8	10.4	141	0.42	40	14	339	509	8.96	3.07	0.26	0.26	0.00	0.00

Individual CBC results – Low Diet Group, Month 2

Cat	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLAT	SEGN	LYMPH	MONO	EOSIN	BASO	BAND
Fox	17.0	9.4	131	0.36	39	14	361	565	7.80	8.00	0.50	0.70	0.00	0.00
Ali	7.9	8.4	114	0.36	43	14	314	n/a	5.30	2.10	0.30	0.20	0.00	0.00
Rota	11.2	7.0	99	0.31	45	14	319	506	4.30	4.00	0.40	2.50	0.00	0.00
Olly	10.6	8.5	115	0.33	39	14	348	471	4.70	5.00	0.20	0.70	0.00	0.00
Meg	11.8	8.4	101	0.29	35	12	347	674	5.80	4.10	0.50	1.40	0.00	0.00
Gunna	15.0	10.2	147	0.41	41	14	355	845	5.60	8.40	0.30	0.80	0.00	0.00
Nonu	12.1	9.7	141	0.49	51	15	287	n/a	7.10	3.90	0.50	0.50	0.00	0.00
Caro	13.7	9.1	129	0.44	48	14	297	504	6.70	5.90	0.40	0.70	0.00	0.00
Red	12.6	8.8	120	0.35	40	14	345	487	6.00	5.20	0.50	0.90	0.00	0.00

Individual CBC results – Medium Diet Group, Month 2

Cat	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLAT	SEGN	LYMPH	MONO	EOSIN	BASO	BAND
Yale	17.4	9.9	139	0.40	40	14	351	635	9.20	6.80	0.50	0.90	0.00	0.00
Nemo	8.5	8.8	131	0.39	44	15	339	443	5.30	2.20	0.50	0.50	0.00	0.00
Pepsi	16.5	8.9	125	0.36	40	14	350	579	6.90	8.10	0.30	1.20	0.00	0.00
Davey	13.0	10.1	150	0.44	43	15	345	319	5.20	6.90	0.30	0.70	0.00	0.00
Badal	8.5	7.9	101	0.29	37	13	347	551	4.70	3.10	0.30	0.40	0.00	0.00
Zaba	9.9	7.7	108	0.31	40	14	351	722	5.70	3.30	0.40	0.50	0.00	0.00
Sione	11.8	9.7	138	0.38	39	14	362	640	4.20	6.80	0.20	0.50	0.00	0.00
Pandy	11.8	8.3	114	0.34	41	14	334	610	5.80	4.80	0.40	0.80	0.00	0.00
Molly	14.8	7.8	104	0.30	39	13	347	362	9.30	3.80	0.60	1.00	0.00	0.00

Individual CBC results – High Diet Group, Month 2

Cat	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLAT	SEGN	LYMPH	MONO	EOSIN	BASO	BAND
Muse	19.5	9.4	138	0.47	50	15	293	383	12.30	5.30	0.80	1.20	0.00	0.00
Carta	9.3	9.4	131	0.39	41	14	340	n/a	4.90	3.30	0.30	0.70	0.00	0.00
Mini	9.7	6.7	95	0.29	43	14	332	504	5.60	3.00	0.40	0.70	0.00	0.00
Nico	16.3	8.1	121	0.36	44	15	340	394	11.20	3.90	0.70	0.50	0.00	0.00
Beau	11.0	8.8	118	0.33	38	13	354	n/a	7.40	2.80	0.60	0.30	0.00	0.00
Toko	9.0	8.8	122	0.37	42	14	331	594	5.10	1.90	0.50	1.50	0.00	0.00
Thys	10.0	9.4	122	0.35	38	13	348	n/a	4.90	4.00	0.40	0.70	0.00	0.00
Richi	11.4	9.2	124	0.36	39	13	347	384	5.10	5.10	0.30	0.80	0.00	0.00
Jaz	12.2	10.5	143	0.44	42	14	326	538	7.00	4.30	0.40	0.60	0.00	0.00

Individual CBC results – Low Diet Group, Month 3

Cat	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLAT	SEGN	LYMPH	MONO	EOSIN	BASO	BAND
Fox	11.9	8.5	119	0.32	38	14	368	552	5.20	5.80	0.40	0.50	0.00	0.00
Ali	8.5	7.9	111	0.32	41	14	344	n/a	5.40	2.60	0.30	0.30	0.00	0.00
Rota	15.4	7.4	108	0.31	42	15	344	311	8.00	4.20	0.60	2.60	0.00	0.00
Olly	11.0	8.6	115	0.32	37	13	361	n/a	4.80	5.10	0.30	0.80	0.00	0.00
Meg	13.4	9.5	115	0.32	33	12	362	7.3	6.70	4.80	0.40	1.50	0.00	0.00
Gunna	16.3	9.0	128	0.36	40	14	358	862	7.70	7.70	0.30	0.70	0.00	0.00
Nonu	11.2	9.3	138	0.43	47	15	318	n/a	7.50	3.00	0.30	0.30	0.00	0.00
Caro	13.8	9.1	133	0.43	47	15	309	602	6.50	6.50	0.30	0.60	0.00	0.00
Red	8.0	8.2	113	0.32	39	14	359	303	3.80	3.00	0.40	0.70	0.00	0.00

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Individual CBC results – Diet Medium, Month 3

Cat	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLAT	SEGN	LYMPH	MONO	EOSIN	BASO	BAND
Yale	14.4	9.5	135	0.39	42	14	344	568	7.78	5.47	0.29	0.86	0.00	0.00
Nemo	12.0	8.8	134	0.37	42	15	361	n/a	7.80	3.12	0.48	0.60	0.00	0.00
Pepsi	17.5	8.5	117	0.34	40	14	348	472	11.20	5.08	0.35	0.88	0.00	0.00
Davey	11.6	9.1	135	0.38	42	15	354	n/a	5.57	5.22	0.23	0.58	0.00	0.00
Badal	7.1	7.8	101	0.29	37	13	354	n/a	4.26	2.34	0.14	0.36	0.00	0.00
Zaba	9.9	8.0	110	0.31	39	14	357	714	6.04	3.07	0.40	0.40	0.00	0.00
Sione	8.6	8.8	127	0.34	38	15	378	670	3.53	4.39	0.17	0.52	0.00	0.00
Pandy	14.9	7.5	100	0.29	38	13	348	699	7.00	4.77	0.60	2.53	0.00	0.00
Molly	15.1	8.3	115	0.32	38	14	361	n/a	9.97	3.93	0.45	0.76	0.00	0.00

Individual CBC results – High Diet Group, Month 3

Cat	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLAT	SEGN	LYMPH	MONO	EOSIN	BASO	BAND
Muse	26.6	9.2	136	0.48	52	15	286	n/a	17.60	6.70	0.80	1.60	0.00	0.00
Carta	8.6	9.8	138	0.40	41	14	347	n/a	4.40	2.20	0.30	0.90	0.00	0.00
Mini	13.0	7.8	113	0.34	44	15	332	458	7.90	3.90	0.40	0.80	0.00	0.00
Nico	17.1	8.0	116	0.34	42	15	344	396	11.30	4.60	0.50	0.70	0.00	0.00
Beau	11.5	8.9	116	0.33	38	13	347	n/a	8.10	2.90	0.20	0.30	0.00	0.00
Toko	14.8	8.9	121	0.35	39	14	347	691	11.10	1.80	0.60	1.30	0.00	0.00
Thys	10.6	9.4	125	0.36	38	13	350	n/a	6.50	3.40	0.20	0.50	0.00	0.00
Richi	12.6	9.2	123	0.34	37	13	360	n/a	6.80	4.90	0.30	0.60	0.00	0.00
Jaz	11.8	9.9	136	0.41	41	14	333	n/a	7.80	3.30	0.20	0.50	0.00	0.00

Individual CBC results – Low Diet Group, Month 4

Cat	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLAT	SEGN	LYMPH	MONO	EOSIN	BASO	BAND
Fox	12.1	9.0	123	0.34	38	14	363	505	5.57	5.45	0.61	0.48	0.00	0.00
Ali	15.5	9.1	125	0.38	42	14	327	n/a	13.49	1.24	0.47	0.31	0.00	0.00
Rota	12.7	7.8	107	0.33	42	14	324	407	6.73	3.18	0.51	2.29	0.00	0.00
Olly	9.0	8.9	116	0.33	37	13	350	353	4.32	3.87	0.27	0.54	0.00	0.00
Meg	10.6	8.8	104	0.30	34	12	349	760	6.04	3.18	0.42	0.95	0.00	0.00
Gunna	17.2	9.7	136	0.39	40	14	351	757	8.08	7.74	0.69	0.69	0.00	0.00
Nonu	13.8	10.2	144	0.50	49	14	289	n/a	9.94	2.90	0.55	0.41	0.00	0.00
Caro	12.5	8.9	128	0.43	48	14	300	n/a	6.50	4.88	0.50	0.63	0.00	0.00
Red	9.9	9.3	127	0.36	39	14	351	n/a	4.26	4.06	0.59	0.99	0.00	0.00

Individual CBC results – Medium Diet Group, Month 4

Cat	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLAT	SEGN	LYMPH	MONO	EOSIN	BASO	BAND
Yale	14.7	9.6	134	0.38	40	14	350	790	8.23	5.15	0.44	0.88	0.00	0.00
Nemo	10.6	10.4	154	0.49	47	15	314	n/a	6.78	2.33	0.74	0.74	0.00	0.00
Pepsi	19.9	8.9	119	0.33	37	13	363	644	8.76	8.76	0.60	1.79	0.00	0.00
Davey	15.6	9.5	135	0.38	41	14	352	450	8.27	6.24	0.47	0.62	0.00	0.00
Badal	7.2	7.0	87	0.25	36	12	345	591	4.46	2.16	0.29	0.29	0.00	0.00
Zaba	11.5	8.5	113	0.32	38	13	352	n/a	7.25	3.34	0.58	0.35	0.00	0.00
Sione	9.9	8.6	122	0.33	39	14	369	595	3.66	4.85	0.20	1.19	0.00	0.00
Pandy	14.4	8.4	106	0.30	35	13	356	709	8.35	3.46	0.58	2.02	0.00	0.00
Molly	14.8	8.4	111	0.32	38	13	351	n/a	11.10	2.52	0.59	0.59	0.00	0.00

Individual CBC results – High Diet Group, Month 4

Cat	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLAT	SEGN	LYMPH	MONO	EOSIN	BASO	BAND
Muse	33.0	9.8	143	0.46	47	15	310	n/a	28.38	2.97	0.99	0.33	0.00	0.33
Carta	12.8	10.1	139	0.40	39	14	348	n/a	8.96	2.30	0.64	0.90	0.00	0.00
Mini	9.5	7.5	107	0.32	42	14	339	n/a	5.32	3.14	0.38	0.67	0.00	0.00
Nico	16.1	8.7	123	0.37	42	14	336	n/a	10.79	4.03	0.64	0.64	0.00	0.00
Beau	9.7	9.4	124	0.35	38	13	350	n/a	5.72	3.01	0.49	0.49	0.00	0.00
Toko	10.9	9.0	123	0.37	42	14	329	758	7.63	1.64	0.55	1.09	0.00	0.00
Thys	12.0	10.5	137	0.40	38	13	343	n/a	7.56	3.48	0.36	0.60	0.00	0.00
Richi	14.1	9.7	124	0.35	36	13	354	465	8.32	4.37	0.56	0.86	0.00	0.00
Jaz	11.4	10.7	144	0.44	42	14	325	n/a	7.18	3.19	0.46	0.57	0.00	0.00

Individual CBC results – Diet Low, Month 5

Cat	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLAT	SEGN	LYMPH	MONO	EOSIN	BASO	BAND
Fox	10.7	8.6	117	0.32	37	14	366	n/a	5.35	4.49	0.43	0.43	0.00	0.00
Ali	8.0	8.4	114	0.35	42	14	329	n/a	5.12	2.08	0.40	0.40	0.00	0.00
Rota	25.3	7.7	106	0.32	41	14	332	559	16.70	4.05	1.01	3.54	0.00	0.00
Olly	12.7	8.7	114	0.32	37	13	353	n/a	5.72	5.08	0.51	1.40	0.00	0.00
Meg	11.5	9.5	113	0.32	34	12	352	720	6.79	3.22	0.35	1.15	0.00	0.00
Gunna	15.5	9.9	138	0.39	40	14	351	811	6.20	7.60	0.62	1.09	0.00	0.00
Nonu	13.2	9.0	131	0.45	50	15	292	n/a	7.92	3.83	0.66	0.66	0.00	0.00
Caro	13.8	8.8	126	0.42	48	14	299	n/a	6.90	5.52	0.55	0.83	0.00	0.00
Red	11.0	8.4	115	0.32	38	14	361	490	5.50	3.74	0.66	1.10	0.00	0.00

Individual CBC results – Medium Diet Group, Month 5

Cat	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLAT	SEGN	LYMPH	MONO	EOSIN	BASO	BAND
Yale	15.1	9.6	133	0.37	39	14	356	n/a	7.85	5.59	0.76	0.91	0.00	0.00
Nemo	9.3	9.5	142	0.38	40	15	378	n/a	5.30	2.70	0.74	0.56	0.00	0.00
Pepsi	17.9	8.4	110	0.32	38	13	347	661	8.41	7.70	0.54	1.25	0.00	0.00
Davey	13.0	9.8	142	0.39	40	14	360	n/a	7.02	5.07	0.39	0.39	0.00	0.00
Badal	7.3	7.6	97	0.27	35	13	362	557	4.31	2.34	0.29	0.37	0.00	0.00
Zaba	9.4	9.5	127	0.36	38	13	349	n/a	5.26	3.20	0.47	0.47	0.00	0.00
Sione	10.6	9.0	126	0.35	39	14	362	n/a	5.51	4.03	0.42	0.64	0.00	0.00
Pandy	13.9	8.8	110	0.34	38	13	327	n/a	7.65	4.17	0.56	1.53	0.00	0.00
Molly	13.9	8.6	114	0.34	39	13	340	n/a	8.48	3.75	0.70	0.97	0.00	0.00

Individual CBC results – High Diet Group, Month 5

Cat	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLAT	SEGN	LYMPH	MONO	EOSIN	BASO	BAND
Muse	18.6	9.4	140	0.49	52	15	287	n/a	10.23	6.14	0.93	1.30	0.00	0.00
Carta	14.4	9.1	128	0.37	40	14	351	n/a	7.92	3.74	0.72	2.02	0.00	0.00
Mini	11.3	7.6	108	0.31	41	14	346	n/a	6.55	3.62	0.45	0.68	0.00	0.00
Nico	15.7	9.0	128	0.38	42	14	340	365	9.58	4.55	0.63	0.94	0.00	0.00
Beau	11.8	9.9	134	0.38	38	14	354	n/a	7.67	2.95	0.59	0.59	0.00	0.00
Toko	16.1	8.6	121	0.35	41	14	343	574	10.63	3.06	0.97	1.45	0.00	0.00
Thys	11.6	10.1	132	0.38	38	13	348	n/a	6.03	4.52	0.46	0.58	0.00	0.00
Richi	12.3	9.6	123	0.35	37	13	350	n/a	6.15	4.92	0.49	0.74	0.00	0.00
Jaz	11.7	10.6	142	0.45	42	13	317	n/a	6.55	4.10	0.35	0.59	0.00	0.00

Individual CBC results – Low Diet Group, Month 6

Cat	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLAT	SEGN	LYMPH	MONO	EOSIN	BASO	BAND
Fox	9.1	8.8	119	0.33	38	14	356	481	4.73	3.46	0.64	0.27	0.00	0.00
Ali	8.0	9.0	121	0.38	42	13	318	n/a	4.88	2.24	0.56	0.32	0.00	0.00
Rota	16.8	7.7	104	0.33	43	14	316	399	9.58	2.52	1.01	3.70	0.00	0.00
Olly	12.9	8.4	112	0.32	38	13	346	465	5.42	4.52	0.52	2.45	0.00	0.00
Meg	12.5	8.6	99	0.29	34	12	341	775	8.38	2.13	0.50	1.50	0.00	0.00
Gunna	18.7	9.1	125	0.36	40	14	344	742	9.91	6.92	0.94	0.94	0.00	0.00
Nonu	14.3	8.6	123	0.41	48	14	301	n/a	9.87	3.00	0.72	0.72	0.00	0.00
Caro	13.8	8.4	122	0.36	43	15	339	378	6.76	5.66	0.69	0.69	0.00	0.00
Red	6.9	8.7	118	0.35	40	14	340	437	3.17	2.69	0.55	0.48	0.00	0.00

Individual CBC results – Medium Diet Group, Month 6

Cat	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLAT	SEGN	LYMPH	MONO	EOSIN	BASO	BAND
Yale	16.9	9.6	133	0.41	43	14	324	647	9.30	5.75	0.68	1.01	0.00	0.00
Nemo	8.0	8.5	125	0.36	42	15	350	486	5.44	1.20	1.12	0.24	0.00	0.00
Pepsi	17.5	8.8	113	0.32	37	13	350	575	9.63	5.95	0.88	1.05	0.00	0.00
Davey	12.3	9.1	129	0.37	40	14	353	380	6.40	4.80	0.62	0.49	0.00	0.00
Badal	8.2	8.0	101	0.30	37	13	342	449	5.08	2.46	0.33	0.33	0.00	0.00
Zaba	15.9	8.7	115	0.33	38	13	350	n/a	11.45	3.34	0.80	0.32	0.00	0.00
Sione	8.8	8.7	122	0.35	40	14	349	402	3.96	3.87	0.44	0.53	0.00	0.00
Pandy	14.1	9.0	114	0.34	38	13	339	761	8.88	3.10	0.71	1.41	0.00	0.00
Molly	15.9	8.4	110	0.32	38	13	343	n/a	11.13	3.18	0.80	0.80	0.00	0.00

Individual CBC results – High Diet Group, Month 6

Cat	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLAT	SEGN	LYMPH	MONO	EOSIN	BASO	BAND
Muse	22.6	8.4	125	0.43	51	15	293	n/a	14.69	5.65	1.13	1.13	0.00	0.00
Carta	13.0	8.5	117	0.35	42	14	331	n/a	6.50	3.12	0.65	2.73	0.00	0.00
Mini	9.5	7.0	99	0.30	43	14	329	450	5.99	2.28	0.67	0.57	0.00	0.00
Nico	18.3	8.9	125	0.36	41	14	343	n/a	12.63	4.03	0.92	0.73	0.00	0.00
Beau	9.8	8.7	118	0.34	39	14	350	n/a	5.98	2.84	0.49	0.49	0.00	0.00
Toko	9.0	8.8	121	0.37	42	14	325	588	6.21	1.17	0.81	0.81	0.00	0.00
Thys	11.3	9.3	122	0.36	39	13	338	n/a	5.88	3.96	0.68	0.79	0.00	0.00
Richi	12.7	9.6	122	0.36	37	13	343	n/a	7.87	3.43	0.64	0.76	0.00	0.00
Jaz	11.2	10.5	139	0.48	46	13	287	537	5.82	4.26	0.45	0.67	0.00	0.00

Appendix 1.7   Individual Biochemistry results

Key: Below Reference Value | Above Reference Value

Low Diet Group, Month 0

Cat	AGR	ALB	ALP	ALT	AST	BIL	CA	CRE	CHOL	CK	CL	GLO	K	PO4	NA	TP	URE
Fox	1.2	38	25	31	17	1	2.41	96	4.2	160	116	33	4.2	1.51	157	71	9.2
Ali	0.9	35	24	33	15	<1	2.24	122	5.1	189	118	38	4.2	1.13	155	73	9.8
Rota	0.9	34	37	73	33	1	2.33	106	3.6	232	117	38	4.8	1.29	156	72	9.7
Olly	1.0	37	15	52	22	1	2.33	87	5.0	127	115	38	4.3	1.41	153	75	7.5
Meg	0.9	34	17	40	29	<1	2.29	84	4.6	148	115	36	4.7	1.60	154	70	9.5
Gunna	0.8	34	24	43	23	<1	2.24	90	2.9	189	113	41	5.5	1.81	152	75	8.1
Nonu	0.6	31	33	32	16	1	2.30	109	2.7	112	114	53	4.3	1.20	152	84	9.0
Caro	0.8	35	25	40	24	<1	2.40	103	3.5	431	113	44	5.0	1.57	154	79	9.6
Red	0.8	35	22	46	16	<1	3.39	79	3.9	149	115	44	4.5	1.57	152	79	7.9

Individual Biochemistry results – Medium Diet Group, Month 0

Cat	AGR	ALB	ALP	ALT	AST	BIL	CA	CRE	CHOL	CK	CL	GLO	K	PO4	NA	TP	URE
Yale	0.8	34	42	50	20	<1	2.40	131	3.8	287	122	45	5.0	1.62	162	79	11.4
Nemo	1.0	38	29	42	16	<1	2.51	108	3.8	123	113	37	4.5	1.57	155	75	8.8
Pepsi	0.8	33	26	42	24	1	2.23	112	2.9	186	114	40	4.7	1.70	154	73	9.9
Davey	1.0	39	29	49	20	1	2.51	126	4.5	151	113	39	4.1	1.77	155	78	9.1
Badal	0.6	34	18	38	18	0	2.27	110	2.1	207	113	55	4.5	1.61	152	89	8.3
Zaba	0.8	32	27	42	19	1	2.14	101	3.4	157	115	40	5.4	1.94	152	72	8.2
Sione	1.0	38	19	56	23	1	2.39	94	4.0	114	116	39	4.3	1.14	152	77	8.5
Pandy	0.9	37	24	40	20	<1	2.41	114	3.6	497	114	39	4.4	1.31	153	76	10.4
Molly	0.6	32	32	69	47	<1	2.20	90	2.5	163	112	52	4.6	1.37	152	84	7.8

Individual Biochemistry results – High Diet Group, Month 0

Cat	AGR	ALB	ALP	ALT	AST	BIL	CA	CRE	CHOL	CK	CL	GLO	K	PO4	NA	TP	URE
Muse	0.6	30	19	55	24	<1	2.18	94	2.1	149	114	47	4.7	1.47	151	77	9.8
Carta	0.7	34	26	35	18	<1	2.29	134	3.5	147	113	47	4.4	1.57	153	81	9.1
Mini	0.8	32	27	48	29	<1	2.27	87	3.7	182	114	39	4.6	1.61	153	71	9.0
Nico	0.7	33	20	30	17	<1	2.40	117	2.8	169	115	48	4.6	1.33	154	81	6.8
Beau	0.8	36	30	57	24	<1	2.28	112	4.1	373	113	45	4.5	1.40	153	81	7.8
Toko	0.9	35	23	44	22	1	2.25	85	2.7	150	114	40	4.8	1.40	153	75	9.4
Thys	0.8	34	20	38	17	1	2.23	103	4.0	135	116	44	5.0	1.68	152	78	8.4
Richi	0.6	31	22	50	23	<1	2.27	121	4.1	159	114	48	5.3	1.64	153	79	8.7
Jaz	0.8	36	29	50	26	<1	2.31	107	3.0	231	111	44	4.5	1.67	153	80	8.5

Individual Biochemistry results – Low Diet Group, Month 6

Cat	AGR	ALB	ALP	ALT	AST	BIL	CA	CRE	CHOL	CK	CL	GLO	K	PO4	NA	TP	URE
Fox	1.2	38	20	36	31	1	2.45	101	3.6	180	118	32	4.6	1.41	152	70	7.5
Ali	1.0	35	25	37	25	1	2.23	119	4.4	209	119	36	4.6	1.38	151	71	9.8
Rota	0.9	36	44	73	34	1	2.43	82	4.3	381	119	38	5.4	1.94	153	74	9.7
Olly	1.1	34	13	48	34	<1	2.25	91	4.4	158	119	32	4.3	1.46	153	66	6.6
Meg	1.0	29	15	47	55	<1	2.10	90	3.3	338	118	29	4.9	1.73	150	58	6.8
Gunna	0.8	33	30	46	33	1	2.48	84	3.2	373	114	44	5.7	1.86	152	77	8.1
Nonu	0.7	33	44	44	26	1	2.34	87	2.6	155	117	48	4.7	1.54	150	81	7.9
Caro	0.9	33	30	51	27	1	2.32	121	2.4	284	122	36	5.6	1.56	155	69	8.2
Red	0.9	34	22	52	26	<1	2.33	83	2.6	141	119	36	4.8	1.96	151	70	6.9

Individual Biochemistry results – Medium Diet Group, Month 6

Cat	AGR	ALB	ALP	ALT	AST	BIL	CA	CRE	CHOL	CK	CL	GLO	K	PO4	NA	TP	URE
Yale	0.9	31	45	59	31	1	2.21	151	3.3	191	120	36	5.7	1.52	151	67	9.6
Nemo	0.9	35	26	44	24	1	2.45	115	2.8	167	117	41	4.3	1.36	151	76	7.0
Pepsi	0.9	32	29	40	23	1	2.29	110	2.9	163	120	37	4.9	1.38	154	69	7.4
Davey	1.1	40	25	53	23	<1	2.54	96	5.8	124	118	36	4.6	1.55	152	76	7.5
Badal	0.6	34	22	58	25	<1	2.44	133	2.5	144	118	53	4.7	1.39	152	87	7.3
Zaba	0.7	24	22	27	31	<1	2.05	99	2.8	181	121	35	4.4	1.40	151	59	9.1
Sione	0.9	34	15	35	25	1	2.33	93	3.6	152	120	36	4.8	1.29	151	70	6.4
Pandy	0.9	34	2	35	22	<1	2.31	111	3.3	214	119	39	4.7	1.08	152	73	7.2
Molly	0.5	27	21	34	28	1	2.20	102	2.7	208	118	50	4.9	1.29	150	77	7.1

Individual Biochemistry results – High Diet Group, Month 6

Cat	AGR	ALB	ALP	ALT	AST	BIL	CA	CRE	CHOL	CK	CL	GLO	K	PO4	NA	TP	URE
Muse	0.8	33	21	63	31	<1	2.31	74	2.4	367	116	42	4.7	1.75	151	75	12.0
Carta	0.8	33	24	41	24	1	2.30	94	4.4	158	118	43	4.5	1.72	151	76	10.3
Mini	0.9	31	25	62	41	<1	2.27	89	2.8	286	120	33	4.5	1.59	153	64	9.3
Nico	0.8	35	18	36	22	<1	2.41	87	3.2	191	116	43	4.8	1.72	151	78	9.4
Beau	0.8	38	30	83	36	1	2.36	102	4.7	512	119	45	4.7	1.32	153	83	8.3
Toko	0.9	31	22	60	46	1	2.11	88	2.9	444	117	35	4.8	1.57	151	66	8.6
Thys	0.8	32	16	45	25	1	2.23	108	3.9	187	120	39	5.0	1.48	151	71	9.0
Richi	0.8	33	23	57	29	<1	2.34	107	5.3	171	118	41	4.9	1.51	152	74	9.0
Jaz	1.1	37	34	69	37	<1	2.29	102	2.5	186	116	35	5.0	1.60	152	72	8.0

Appendix 1.8   Urinalysis results

Key:

	Below Reference Value		Above Reference Value	BIL	bilirubin	ERY	Erythrocytes
GLU	Glucose	Hb	Haemoglobin	KET	Ketones	HUV	Spun Volume (mLs)
LEU	Leukocytes	NIT	Nitrites	PROT	Protein	UBA	Urinary bacteria
UBG	Urobilinogen	UCAS	Urinary casts	UCOL	Urine colour	UCRY	Crystals seen
UEPI	Epithelial cells	URBC	Red Blood cells	USCOL	Supernatant colour	USG	Urine specific gravity
UTUR	Urine turbidity	UWBC	White blood cells				

Dipstick urinalysis interpretation scale
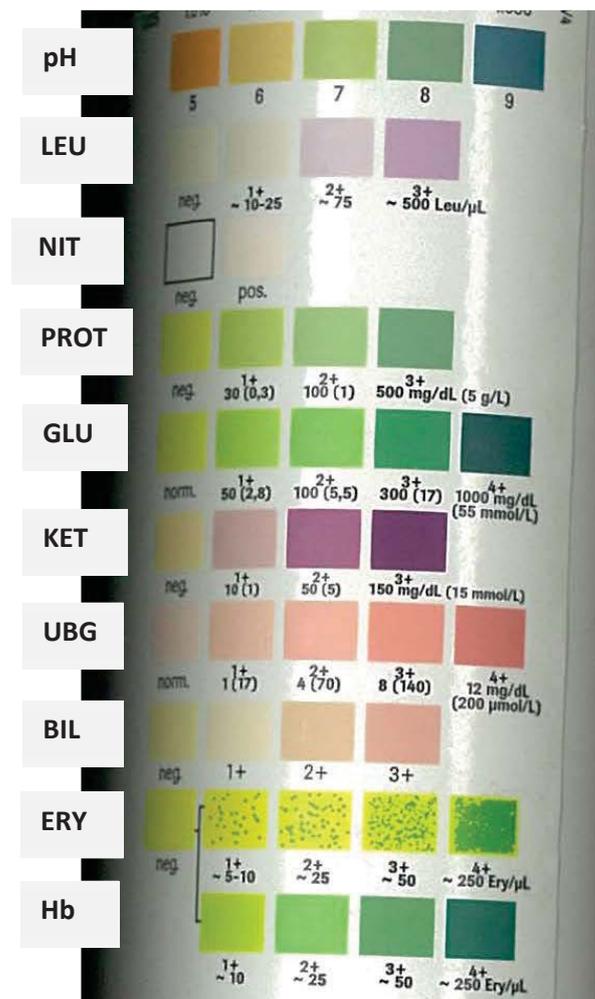
Commercial Urinalysis – Low Diet group, Month 0

Diet	Cat	HUV	UBA	UCAS	UCOL	UCRY	UEPI	Method	URBC	USCOL	USG	UTUR	UWBC
Low	Fox	1	None	None	Pale Yellow	None	None	Cysto	None	Pale Yellow	1.022	Clear	None
Low	Ali	1	None	None	Pale Yellow	None	None	Cysto	None	Pale Yellow	1.039	Hazy	None
Low	Rota	1	None	None	Pale Yellow	None	None	Cysto	Occasional	Pale Yellow	1.017	Clear	None
Low	Olly	1	None	None	Pale Yellow	None	None	Cysto	None	Pale Yellow	1.013	Clear	None
Low	Meg	1	None	None	Pale Yellow	None	None	Cysto	None	Pale Yellow	1.013	Clear	None
Low	Gunna	1	None	None	Pale Yellow	None	None	Cysto	Occasional	Pale Yellow	1.015	Clear	None
Low	Nonu	1	None	None	Pale Yellow	None	Occasional	Cysto	5-10	Pale Yellow	1.031	Hazy	None
Low	Caro	1	None	None	Pale Yellow	None	None	Cysto	None	Pale Yellow	1.033	Hazy	None
Low	Red	1	Occasional	None	Pale Yellow	None	None	Cysto	None	Pale Yellow	1.026	Hazy	None

Commercial Urinalysis – Medium Diet Group, Month 0

Diet	Cat	HUV	UBA	UCAS	UCOL	UCRY	UEPI	Method	URBC	USCOL	USG	UTUR	UWBC
Medium	Yale	1	None	None	Pale Yellow	None	Occasional	Cysto	5-10	Pale Yellow	1.024	Clear	None
Medium	Nemo	1	None	None	Pale Yellow	None	None	Cysto	5-10	Pale Yellow	1.025	Clear	None
Medium	Pepsi	1	+	None	Yellow	None	None	Cysto	Occasional	Yellow	1.040	Hazy	None
Medium	Davey	1	None	None	Pale Yellow	None	None	Cysto	Occasional	Pale Yellow	1.027	Clear	None
Medium	Badal	1	None	None	Pale Yellow	None	None	Cysto	Occasional	Pale Yellow	1.032	Clear	None
Medium	Zaba	1	None	None	Pale Yellow	None	None	Cysto	Occasional	Pale Yellow	1.021	Clear	None
Medium	Sione	1	None	None	Pale Yellow	None	None	Cysto	Occasional	Pale Yellow	1.032	Clear	None
Medium	Pandy	1	None	None	Pale Yellow	None	None	Cysto	None	Pale Yellow	1.025	Clear	None
Medium	Molly	1	None	None	Pale Yellow	None	None	Cysto	None	Pale Yellow	1.031	Hazy	None

Commercial Urinalysis – High Diet Group, Month 0

Diet	Cat	HUV	UBA	UCAS	UCOL	UCRY	UEPI	Method	URBC	USCOL	USG	UTUR	UWBC
High	Muse	1	None	None	Pale Yellow	None	None	Cysto	None	Pale Yellow	1.019	Clear	None
High	Carta	1	None	None	Pale Yellow	None	None	Cysto	None	Pale Yellow	1.036	Hazy	None
High	Mini	1	None	None	Pale Yellow	None	None	Cysto	None	Pale Yellow	1.035	Clear	None
High	Nico	1	None	None	Pale Yellow	+++	None	Cysto	None	Pale Yellow	1.041	Hazy	None
High	Beau	1	None	None	Pale Yellow	None	None	Cysto	None	Pale Yellow	1.016	Clear	None
High	Toko	1	None	None	Pale Yellow	None	None	Cysto	None	Pale Yellow	1.015	Clear	None
High	Thys	1	None	None	Pale Yellow	None	None	Cysto	None	Pale Yellow	1.026	Clear	None
High	Richi	1	None	None	Pale Yellow	None	None	Cysto	Occasional	Pale Yellow	1.018	Clear	None
High	Jaz	1	None	None	Pale Yellow	None	None	Cysto	None	Pale Yellow	1.024	Clear	None

Urine refractometer & dipstick values - Month 0

Diet	Cat	USG	Dipstick measures	
			pH	Additional measures
Low	Fox	1.026	6.5	All negative
Low	Ali	1.043	7	All negative
Low	Rota	1.020	6.5	All negative
Low	Olly	1.017	6	All negative
Low	Meg	1.016	5.5	All negative
Low	Gunna	1.019	7	All negative
Low	Nonu	1.036	6.5	All negative
Low	Caro	1.073	8	All negative
Low	Red	1.030	7	All negative
Medium	Nemo	1.029	5.5	All negative
Medium	Pepsi	1.039	5.5	All negative
Medium	Davey	1.032	6	All negative
Medium	Badal	1.036	5	All negative
Medium	Zaba	1.026	7	All negative
Medium	Sione	1.036	7.5	All negative
Medium	Pandy	1.029	5	All negative
Medium	Molly	1.035	5.5	All negative
Medium	Yale	1.027	7	All negative
High	Muse	1.023	5	All negative
High	Carta	1.040	7	All negative
High	Mini	1.039	7	All negative
High	Nico	1.045	8	PROT +1
High	Beau	1.020	7	All negative
High	Toko	1.019	6.5	All negative
High	Thys	1.031	8	All negative
High	Richi	1.022	7.5	All negative
High	Jaz	1.028	7	All negative

Urine refractometer & dipstick values - Month 2

Diet	Cat	USG	Dipstick measures	
			pH	Additional Measures
Low	Fox	1.037	no measurement	no measurement
Low	Ali	1.043	no measurement	no measurement
Low	Rota	1.040	no measurement	no measurement
Low	Olly	1.042	no measurement	no measurement
Low	Meg	1.037	no measurement	no measurement
Low	Gunna	1.042	no measurement	no measurement
Low	Nonu	1.030	no measurement	no measurement
Low	Caro	1.044	no measurement	no measurement
Low	Red	1.038	no measurement	no measurement
Medium	Yale	1.027	no measurement	no measurement
Medium	Nemo	1.028	no measurement	no measurement
Medium	Pepsi	1.046	no measurement	no measurement
Medium	Davey	1.060	no measurement	no measurement
Medium	Badal	1.037	no measurement	no measurement
Medium	Zaba	1.033	no measurement	no measurement
Medium	Sione	1.036	no measurement	no measurement
Medium	Pandy	1.044	no measurement	no measurement
Medium	Molly	1.042	no measurement	no measurement
High	Muse	1.035	no measurement	no measurement
High	Carta	1.048	no measurement	no measurement
High	Mini	1.056	no measurement	no measurement
High	Nico	1.044	no measurement	no measurement
High	Beau	1.048	no measurement	no measurement
High	Toko	1.033	no measurement	no measurement
High	Thys	1.033	no measurement	no measurement
High	Richi	1.043	no measurement	no measurement
High	Jaz	1.047	no measurement	no measurement

Urine refractometer & dipstick values - Month 3

Diet	Cat	USG	Dipstick Measures	
			pH	Additional Measures
Low	Fox	1.048	6.5	All negative
Low	Ali	1.045	6	All negative
Low	Rota	1.021	5	All negative
Low	Olly	1.046	7	PROT+1. Rest negative
Low	Meg	1.047	7	PROT+1. Rest negative
Low	Gunna	1.042	6.5	PROT+1, Hb+3. Rest negative
Low	Nonu	1.038	6	PROT+1. Rest negative
Low	Caro	1.031	5.5	PROT+1. Rest negative
Low	Red	1.043	6.5	All negative
Medium	Yale	1.044	6	All negative
Medium	Nemo	1.035	7	All negative
Medium	Pepsi	1.042	7.5	All negative
Medium	Davey	1.048	7	PROT+1. Rest negative
Medium	Badal	1.034	6.5	PROT+1. Rest negative
Medium	Zaba	1.025	6.5	All negative
Medium	Sione	1.047	7	PROT+1. Rest negative
Medium	Pandy	1.049	7	PROT+1. Rest negative
Medium	Molly	1.049	7.5	PROT+1. Rest negative
High	Muse	1.048	5	PROT+1. Rest negative
High	Carta	1.047	6.5	PROT+2. Rest negative
High	Mini	1.060	8	PROT+2. Rest negative
High	Nico	1.045	7	PROT+1. Rest negative
High	Beau	1.038	6.5	PROT+1. Rest negative
High	Toko	1.037	7	PROT+1. Rest negative
High	Thys	1.043	6	PROT+1. Rest negative
High	Richi	1.028	7.5	PROT+3. Rest negative
High	Jaz	1.049	7	PROT+1. Rest negative

Urine refractometer & dipstick values - Month 4

Diet	Cat	USG	Dipstick Measures	
			pH	Additional Measures
Low	Fox	1.046	7	PROT+1. Rest negative
Low	Ali	1.023	7	PROT+1, Hb+3. Rest negative
Low	Rota	1.019	5	All negative
Low	Olly	1.042	7.5	PROT+1, Hb+1. Rest negative
Low	Meg	1.032	6	PROT+1. Rest negative
Low	Gunna	1.031	6.5	PROT+1. Rest negative
Low	Nonu	1.030	7	PROT+1, Hb+1. Rest negative
Low	Caro	1.024	6	All negative
Low	Red	1.021	6	PROT+1. Rest negative
Medium	Yale	1.021	6	PROT+1. Rest negative
Medium	Nemo	1.025	7	PROT+1. Rest negative
Medium	Pepsi	1.026	7	PROT+1. Rest negative
Medium	Davey	1.039	7	PROT+1. Rest negative
Medium	Badal	1.018	7	PROT+1. Rest negative
Medium	Zaba	1.015	5	All negative
Medium	Sione	1.030	7	PROT+1, Hb+2. Rest negative
Medium	Pandy	1.016	7	PROT+1. Rest negative
Medium	Molly	1.046	7	PROT+1. Rest negative
High	Muse	1.040	5.5	PROT+1. Rest negative
High	Carta	1.045	6	PROT+1. Rest negative
High	Mini	1.048	7	PROT+1. Rest negative
High	Nico	1.044	6.5	PROT+1. Rest negative
High	Beau	1.021	7	PROT+1, Hb+2. Rest negative
High	Toko	1.029	7	PROT+1, Hb+1. Rest negative
High	Thys	1.026	6.5	PROT+1. Rest negative
High	Richi	1.018	6	Rest negative
High	Jaz	1.040	5	PROT+1. Rest negative

Urine refractometer & dipstick values - Month 5

Diet	Cat	USG	Dipstick Measures	
			pH	Additional Measures
Low	Fox	1.042	7	Hb+2. Rest negative
Low	Ali	1.039	6	All negative
Low	Rota	1.022	7.5	All negative
Low	Olly	1.036	6	All negative
Low	Meg	1.036	7	PROT+1, Hb+3. Rest negative
Low	Gunna	1.033	7	PROT+1. Rest negative
Low	Nonu	1.038	5	PROT+1. Rest negative
Low	Caro	1.037	6.5	PROT+1. Rest negative
Low	Red	1.046	5.5	PROT+1. Rest negative
Medium	Yale	1.028	8	All negative
Medium	Nemo	1.037	6	PROT+1. Rest negative
Medium	Pepsi	1.028	7	PROT+1. Rest negative
Medium	Davey	1.043	6.5	All negative
Medium	Badal	1.033	6	PROT+1. Rest negative
Medium	Zaba	1.043	7	PROT+1. Rest negative
Medium	Sione	1.046	7	PROT+1. Rest negative
Medium	Pandy	1.044	8	PROT+1. Rest negative
Medium	Molly	1.064	7	PROT+1. Rest negative
High	Muse	1.035	6	All negative
High	Carta	1.044	6.5	All negative
High	Mini	1.044	6.5	PROT+1. Rest negative
High	Nico	1.040	6	All negative
High	Beau	1.025	6	All negative
High	Toko	1.020	7	PROT+1. Rest negative
High	Thys	1.039	7	PROT+1. Rest negative
High	Richi	1.037	7	All negative
High	Jaz	1.041	7	PROT+1. Rest negative

Urine refractometer & dipstick values - Month 6

Diet	Cat	USG	Dipstick Measures	
			pH	Additional Measures
Low	Fox	1.039	7	All negative
Low	Ali	1.034	7	PROT+1. Rest negative
Low	Rota	1.023	5.5	All negative
Low	Olly	1.017	8	All negative
Low	Meg	1.036	7	PROT+1. Rest negative
Low	Gunna	1.038	7	All negative
Low	Nonu	1.026	8	All negative
Low	Caro	1.038	6	PROT+1. Rest negative
Low	Red	1.039	6	PROT+1, Hb+3. Rest negative
Medium	Yale	1.022	7	All negative
Medium	Nemo	1.020	6	All negative
Medium	Pepsi	1.017	7	All negative
Medium	Davey	no sample		
Medium	Badal	1.024	8	PROT+1. Rest negative
Medium	Zaba	1.023	6	PROT+1. Rest negative
Medium	Sione	1.020	8	ERY+3. Rest negative
Medium	Pandy	1.028	6	PROT+1, ERY+3. Rest negative
Medium	Molly	1.044	7	PROT+1. Rest negative
High	Muse	1.022	7.5	Rest negative
High	Carta	1.028	6	PROT+1. Rest negative
High	Mini	1.036	7	PROT+1. Rest negative
High	Nico	1.045	6.5	PROT+1. Rest negative
High	Beau	1.017	5.5	All negative
High	Toko	no sample		
High	Thys	1.018	6	All negative
High	Richi	1.017	5	All negative
High	Jaz	1.025	8	PROT+1. Rest negative

Appendix 1.9   Individual Mean Blood Pressure

Key:		Below Reference Value		Above Reference Value

02/04/14 (Day 167)

Date	Diet	Cat	SBP	DBP	MAP	HR
2/04/2014	Low	Meg	128.00	108.00	114.67	187.00
2/04/2014	Low	Nonu	133.50	106.50	115.75	139.50
2/04/2014	Low	Caro	167.00	117.33	134.00	101.00
2/04/2014	Low	Olly	111.50	89.50	96.50	117.50
2/04/2014	Low	Rota	114.67	89.67	98.33	143.33
2/04/2014	Low	Ali	126.67	101.67	110.00	139.33
2/04/2014	Low	Gunna	113.00	67.33	82.33	153.33
2/04/2014	Low	Fox	131.67	104.67	113.67	127.00
2/04/2014	Low	Red	124.00	84.67	97.67	105.67
2/04/2014	Medium	Davey	164.75	84.00	110.75	147.75
2/04/2014	Medium	Pepsi	165.67	152.67	156.33	183.00
2/04/2014	Medium	Yale	140.67	102.00	114.67	173.67
2/04/2014	Medium	Zaba	137.00	97.67	111.00	177.33
2/04/2014	Medium	Nemo	143.67	83.00	103.33	94.33
2/04/2014	Medium	Molly	195.50	121.00	146.00	150.00
2/04/2014	Medium	Pandy	116.67	81.33	93.00	133.33
2/04/2014	Medium	Sione	170.00	71.50	104.25	107.75
2/04/2014	Medium	Badal	103.00	81.75	88.75	144.75
2/04/2014	High	Carta	150.33	119.33	129.33	108.33
2/04/2014	High	Toko	121.00	75.33	90.33	163.67
2/04/2014	High	Beau	150.25	81.75	104.25	136.00
2/04/2014	High	Mini	120.33	100.00	106.33	131.67
2/04/2014	High	Nico	148.67	59.33	88.67	94.67
2/04/2014	High	Richi	128.75	73.00	91.25	129.50
2/04/2014	High	Jaz	176.50	99.75	125.25	157.25
2/04/2014	High	Muse	132.00	75.33	94.00	133.33
2/04/2014	High	Thys	115.25	90.50	98.50	119.00

Individual Mean Blood Pressure - 09/04/14 (Day 174)

Date	Diet	Cat	SBP	DBP	MAP	HR
9/04/2014	Low	Meg	144.67	126.67	132.67	203.67
9/04/2014	Low	Nonu	129.67	104.33	112.67	129.33
9/04/2014	Low	Caro	148.00	81.00	103.00	136.00
9/04/2014	Low	Olly	178.33	128.00	144.67	121.67
9/04/2014	Low	Rota	138.33	96.00	110.00	174.00
9/04/2014	Low	Ali	168.50	77.00	107.50	108.00
9/04/2014	Low	Gunna	125.67	88.33	101.00	120.00
9/04/2014	Low	Fox	186.50	129.50	148.50	125.00
9/04/2014	Low	Red	178.33	114.00	135.67	112.00
9/04/2014	Medium	Davey	141.00	85.33	103.67	96.00
9/04/2014	Medium	Pepsi	164.00	125.67	138.33	110.00
9/04/2014	Medium	Yale	133.33	99.00	110.33	148.00
9/04/2014	Medium	Zaba	124.00	85.67	98.33	143.67
9/04/2014	Medium	Nemo	121.00	81.00	94.25	129.00
9/04/2014	Medium	Molly	142.33	121.33	128.33	163.33
9/04/2014	Medium	Pandy	138.00	108.00	118.00	118.67
9/04/2014	Medium	Sione	166.67	126.00	139.67	121.67
9/04/2014	Medium	Badal	128.00	115.50	120.00	144.00
9/04/2014	High	Carta	106.00	59.67	75.33	107.00
9/04/2014	High	Toko	113.33	77.00	89.00	197.00
9/04/2014	High	Beau	119.33	71.33	87.33	142.67
9/04/2014	High	Mini	115.33	99.00	104.33	145.33
9/04/2014	High	Nico	150.50	85.25	107.00	99.75
9/04/2014	High	Richi	145.67	92.33	110.00	126.00
9/04/2014	High	Jaz	172.00	116.00	134.67	148.67
9/04/2014	High	Muse	136.67	102.00	113.67	114.33
9/04/2014	High	Thys	119.33	87.00	97.67	128.00

Individual Mean Blood Pressure – 16/04/14 (Day 181)

Date	Diet	Cat	SBP	DBP	MAP	HR
16/04/2014	Low	Meg	96.33	72.33	80.67	156.67
16/04/2014	Low	Nonu	147.33	71.33	96.67	119.67
16/04/2014	Low	Caro	156.33	96.33	116.33	114.33
16/04/2014	Low	Olly	121.67	76.67	91.33	105.00
16/04/2014	Low	Rota	119.00	96.33	104.00	125.00
16/04/2014	Low	Ali	159.33	100.67	120.00	162.33
16/04/2014	Low	Gunna	160.67	125.00	135.67	115.00
16/04/2014	Low	Fox	135.33	70.33	91.67	127.00
16/04/2014	Low	Red	151.33	121.00	131.00	134.67
16/04/2014	Medium	Davey	147.67	102.33	118.00	123.33
16/04/2014	Medium	Pepsi	114.67	60.33	78.33	118.33
16/04/2014	Medium	Yale	141.67	84.00	103.00	167.33
16/04/2014	Medium	Zaba	136.33	93.33	107.67	158.67
16/04/2014	Medium	Nemo	141.33	101.00	114.33	119.00
16/04/2014	Medium	Molly	163.33	96.00	118.33	129.67
16/04/2014	Medium	Pandy	80.00	41.00	54.00	124.00
16/04/2014	Medium	Sione	159.33	76.33	104.00	95.33
16/04/2014	Medium	Badal	136.33	64.00	88.00	125.33
16/04/2014	High	Carta	110.00	89.00	95.67	103.33
16/04/2014	High	Toko	153.33	119.00	130.67	139.33
16/04/2014	High	Beau	133.00	86.33	102.00	120.67
16/04/2014	High	Mini	141.33	109.00	119.67	125.00
16/04/2014	High	Nico	141.00	107.67	118.67	125.00
16/04/2014	High	Richi	148.33	127.33	134.33	124.33
16/04/2014	High	Jaz	177.67	140.00	152.67	142.33
16/04/2014	High	Muse	173.33	120.67	138.00	124.33
16/04/2014	High	Thys	155.00	86.00	108.67	89.33

Appendix 1.10   Individual GFRs

(ml/min/kg)

Diet	ANIMAL	BASELINE	3-MONTHS	6-MONTHS
Low	Fox	4.20	3.45	4.87
Low	Ali	1.76	2.52	1.82
Low	Rota	1.67	2.31	5.91
Low	Olly	2.74	1.68	2.71
Low	Meg	3.14	1.63	2.58
Low	Gunna	2.99	2.49	3.70
Low	Nonu	0.88	1.48	2.79
Low	Caro	5.11	1.59	1.87
Low	Red	1.98	2.36	3.80
Medium	Yale	1.61	1.86	2.04
Medium	Nemo	1.58	1.52	1.80
Medium	Pepsi	1.79	4.58	3.19
Medium	Davey	1.76	3.74	1.56
Medium	Badal	2.23	2.31	1.67
Medium	Zaba	1.24	2.08	3.21
Medium	Sione	2.76	4.54	3.66
Medium	Pandy	1.78	3.82	1.99
Medium	Molly	1.74	4.04	2.92
High	Muse	3.13	5.50	3.54
High	Carta	2.32	1.85	2.79
High	Mini	2.15	3.38	3.75
High	Nico	3.25	3.53	3.20
High	Beau	2.72	3.27	2.30
High	Toko	2.30	2.54	3.33
High	Thys	1.32	1.72	1.67
High	Richi	2.76	3.99	2.14
High	Jaz	2.49	5.63	3.78

Appendix 1.10   Individual GFRs

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