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"STUDY OF A NEW FORMULATION OF FUNCTIONAL FOOD FOR DOGS WITH CHRONIC RENAL AND CARDIOVASCULAR DISEASES"

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Don't be afraid to dream big.

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my special thanks go to my family and my children.

Dedicated to my children

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1. INTRODUCTION

1.1 The urinary system and kidney function

1.1.1 Anatomy and physiology of the urinary system

The urinary system (Figure1) includes:

- two kidneys that make urine and carry out other vital functions
- two ureters that carry urine to the urinary bladder
- One urinary bladder that collects, stores and releases urine
- One urethra that conducts urine from the body

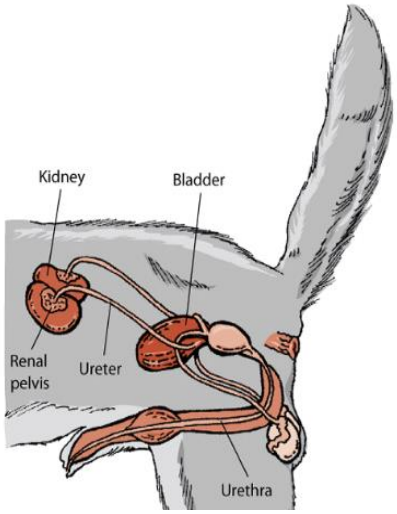


Figure 1 The urinary system in the dog

The urinary system has several important functions. It gets rid of the waste products that are created when food is transformed into energy. It also

maintains the correct balance of water and electrolytes (salts) within the body's cells. Another key function is the production of hormones called erythropoietin and renin, which are important in maintaining healthy blood pressure, producing blood cells, and absorbing salt correctly. Finally, the urinary system processes vitamin D.

The kidneys are paired organs suspended from the dorsal abdominal wall by a peritoneal fold and the blood vessels that serve them. They are located slightly cranial to the mid lumbar region. Because they are separated from the abdominal cavity by their envelopment of peritoneum, they are called retroperitoneal structures. Blood is carried to each kidney by a renal artery, and venous blood is conveyed away from each kidney by a renal vein. The renal artery arises directly from the aorta, and the renal vein empties directly into the caudal vena cava (Reece W. O., 2009).

The kidneys are located in the dorsal part ofthe abdomen, just ventralto and on either side of the first few lumbar vertebrae. The right kidney is more cranial than the left. A thick layer of fat (perirenal fat) usually surrounds the kidneys and helps protect them from pressure exerted by surrounding organs (Colville and Bassert, 2016).

Anatomically, the kidneys consist of an outer region, the cortex covered by a capsule, and an inner region, the medulla, which embraces a centrally situated pelvis (Figure 2).

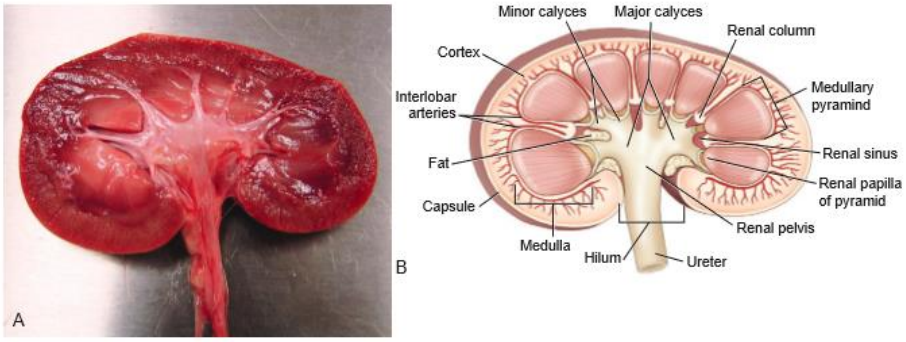


Figure 2 canine kidney: A gross anatomy; B kidney section

The kidneys are responsible for many different aspects of homeostasis and can be divided into three functionally different compartments; the glomerular, the tubular and the interstitial compartments. Some authors also consider the renal vasculature a fourth compartment. The principal function of the kidney is to clear unwanted substances from the body, either by glomerular filtration, or by active secretion into the urine by the tubular system, or both. Examples of unwanted substances are end-products of metabolism such as inorganic phosphate and potassium and exogenous substances such as drugs and toxins. The kidneys are responsible for fluid-, acid/base- and electrolyte homeostasis as well as blood pressure regulation. Moreover, the kidneys perform multiple endocrine functions: interstitial fibroblasts produce erythropoietin (EPO), a hormone needed for production of red blood cells (Kurtz et al., 1989). The juxtaglomerular cells within the kidney produces the hormone renin in response to reduced circulating volume (Kurtz, 2011). The kidneys contain internal

receptors that monitor blood pressure. When blood pressure falls,the kidneys secrete a hormone called renin. The release of renin will start a cascade of reactions that will result in vasoconstriction and the retention of sodium and water. By increasing the fluid volume of the blood, blood pressure will also be increased (Colville and Bassert, 2016).

When blood pressure falls, the renin–angiotensin–aldosterone system responds to bring it back up to normal levels. Within the afferent glomerular arterioles are specialized juxtaglomerular cells that constantly monitor blood pressure within the arterioles. In addition,there is a group of densely packed cells within the ascending limb of the loop of Henle, called the macula densa, that monitors the NaCl concentration of the tubular filtrate. If the juxtaglomerular cells detect a decrease in blood pressure or if the macula densa cells come into contact with less NaCl (either because of a lower NaCl concentration or sluggish flow of filtrate through the tubules) the juxtaglomerular cells will respond by releasing renin. Renin is an enzyme that facilitates the splitting of angiotensin I from angiotensin. Angiotensin I is then converted into angiotensin II by angiotensin-converting enzyme (ACE). Angiotensin II is the active molecule that causes arterial constriction and stimulates the release of aldosterone from the adrenal glands. By increasing sodium and water reabsorption back into the bloodstream, aldosterone will cause an increase in blood volume. As blood volume increases, it will better fill the vascular space and increase blood pressure. In the kidney the inactive metabolite 1, 25 dihydroxycholecalciferol

is transformed into calcitriol, “activated” vitamin D, by 1α-hydroxylase, an enzyme of which the kidney is the most abundant source. Each canine kidney contains several hundred thousand functional units called nephrons. Every nephron consists of a renal corpuscle (glomerulus, mesangium and Bowman´s capsule) and a tubular system, through which urine is transported towards the renal pelvis. The tissue between nephrons and vessels, the interstitium, acts as a support structure for cells. It consists of a highly charged extra-cellular matrix (ECM), which is most prominent in the renal medulla. Proteases (for example matrix metalloproteinases, MMPs) and their inhibitors maintain the delicate equilibrium between ECM synthesis and degradation (Aresu et al., 2011). It is now recognized that the ECM, in addition to its supportive function, also is highly active in cell signaling (Seikrit et al., 2013; Rozario & DeSimone, 2010). Through the renal arteries, the kidneys receive a sizable part (approximately 20%) of cardiac output, especially considering the small size (about 1% of body mass) of these organs. After flowing through interlobar, arcuate and interlobular arteries, blood enters the glomerular tuft, a web of fenestrated capillaries, where filtration takes place. Mesangial cells, which have contractile properties and are thought to contribute to the glomerular filtration function, provide structure for the glomerular capillary loops (Schlondorff, 1987). Glomerular plasma flow and intraglomerular pressure are fine-tuned by hemodynamic actions of the afferent and efferent arterioles at both ends of the glomerular tuft (Brown et al., 1990). Blood is filtrated through three layers; the

fenestrated endothelium, the glomerular basement membrane and the epithelium, through the slit diaphragm of podocytes (Deen et al., 2001). These layers together provide a size- and charge-selective barrier (Brenner et al., 1977). Hormonal, neural and vasoactive substances influence renal blood flow and glomerular filtration. Low molecular weight (LMW) molecules with radii < 20-25 Å (or less than ≈ 25 kDa) are relatively freely filtered, and those with radii >50-55 Å (or weights of more than ≈ 70 kDa) are to a great extent excluded from filtration (D'Amico & Bazzi, 2003; Oliver et al., 1992; Maack et al., 1979).

Apart from size and charge, the sieving coefficient (glomerular permeability) of different substances, especially medium-sized ones, also depends on the flexibility and shape of circulating molecules (Lindstrom et al., 1997; Maack et al., 1979). The fraction of plasma that is filtrated depends on glomerular plasma flow, intraglomerular hydrostatic pressure, and ultrafiltration coefficient Kf (where Kf is the product of filtration barrier permeability and surface area) (Deen et al., 1972). The filtrated plasma (ultrafiltrate, or primary urine) enters Bowman´s capsule, which constitutes the first part of the renal tubular system. The rate of filtration by the glomeruli, or the GFR, is defined as the amount of ultrafiltrate that forms in the nephrons per unit of time. The level of GFR in an individual person or animal with healthy kidneys is set by the metabolic rate (Singer, 2001).

Bowman´s capsule opens up into the proximal tubules, where 66-75% of the ultrafiltrate is reabsorbed, including approximately 60% of filtered sodium, potassium and chloride, 70% of filtered calcium, and 80% of filtered phosphate and bicarbonate (Boron, 2006; Duarte & Watson, 1967; Malnic et al., 1964). Glucose and amino acids, such as cystine, ornithine, lysine and arginine are almost completely reabsorbed in the proximal tubular segment (Silbernagl, 1988). Small filtered proteins such as albumin (most of which is retained from filtration, but filtered in small amounts in healthy individuals), are reabsorbed into proximal tubular cells by megalin-mediated pinocytosis (Vinge et al., 2010; Lazzara & Deen, 2007). In the remaining parts of the tubular system, further concentration of urine and necessary adjustment of acid/base-, mineral- and electrolyte-status occurs. The filtrate then enters the renal pelvis and the urethra for transport to the urinary bladder as urine. Blood that is not filtered in the glomerulus and instead enters the efferent arteriole flows through another, peritubular, capillary system. These capillaries provide oxygen and nutrition to the renal parenchyma, including the tubular system (Beeuwkes & Bonventre, 1975).

1.1.2 Kidney disease

Renal failure, defined as the loss of ability of kidney to maintain osmoregulation and body homeostasis, is one of the serious problems in canines

and about 2-5% of dogs suffer from renal diseases. It is the third cause leading to death in dogs (Lund et al., 1999; Chew et al., 2011a). Renal failure can be caused by decreased blood flow to kidneys (prerenal failure/prerenal azotemia) or disorders that disrupt structures in the kidney (intrinsic renal failure/renal azotemia) or disorders that interfere with elimination of urine from the kidneys (postrenal failure/ postrenal azotemia). This may take the form of acute or chronic renal failure (Harold, 2005). In a recent study in India, pre-renal (20%), renal (57%) and post-renal (23%) causes of renal failures were observed by Tufani et al., (2015). The terminology acute renal failure has recently been modified to acute kidney injury (AKI). Similarly, the chronic renal failure has been modified to chronic kidney disease (CKD), both in the human and the veterinary sciences. The reasons for these modifications are to better describe the disorder since not every kidney injury is associated with a measurable failure and to sensitize clinicians for early identification of the disease (Segev, 2011). The prevalence of CKD is higher in geriatric dogs, with chronic renal insufficiency reaching a peak prevalence of 10% in veterinary clinical patients (Polzin et al., 1992). Tufani et al., (2015) reported 2.58% clinical prevalence of renal failure in dogs. Breed-wise prevalence of renal failure was highest in Labrador followed by German shepherd and Pomeranian. Among renal failure cases, AKI) was observed in 58% (males-63.78% and females-36.21%) and CKD) in 42% (males-54.76% and females-45.24%) cases. The incidence AKI was highest in 4 to 8 years of age (43.10%) and lowest in >8 years of age

(25.86%) whereas CKD was highest in >8 years of age (80.95%) and lowest in <4 years of age (4.76%). Acute renal failure is a rapid decline in renal function, resulting in retention of uremic toxins, as well as fluid, acid-base and electrolyte imbalances is often reversible while chronic renal failure develops slowly and is the end result of irreparable kidney damage (Segev, 2011; Harold, 2005).

Different etiological factors are involved in dogs with renal failure, 47% were found to be idiopathic, 23% urolithiasis, 20% leptospirosis and 10% pyometra (Tufani et al., 2015). Further etiological causes are listed below according to the type of renal failure:

(1) Acute prerenal: - Volume depletion;- Secondary to heart failure

(2) Acute intrinsic/renal failure: - Nephritis (embolic, leptospiral, pyelonephritis and glomerulonephritis); - Nephrosis (ischemia, nephrotoxicity potentially by antibiotic toxicity)

(3) Acute postrenal failure: - Uroabdomen;- Bilateral obstruction as per Chew et al., (2011b).

The development of the disease can be divided into 4 phases: Initiation, Extension, Maintenance and Recovery. After the recovery phase patient either would gain partial or complete kidney function or may develop progressive chronic renal failure (Segev, 2011; Chew et. al, 2011b).

Acute kidney injury (AKI) is defined as an abrupt decline in renal filtration characterized by elevated serum creatinine levels, acute uremia, and changes in urine volume. AKIs affect dogs and cats similar to humans, maybe associated

with one or more of various contributory causes and may vary in severity (Brown et all.,2015; Lee et all.2012).

Renal failure occurs when approximately three fourths of the nephrons of both kidneys cease to function. AKI results from an abrupt decline in renal function and is usually caused by an ischemic or toxic insult to the kidneys, although leptospirosis is reemerging as an important infectious cause of AKI. Ischemic or toxicant-induced injury usually results in damage to the metabolically active epithelial cells of the proximal tubules and thick ascending loop of Henle, causing impaired regulation of water and solute balance. Nephrotoxicants interfere with essential tubular cell functions and cause cellular injury, swelling, and death. Renal ischemia causes cellular hypoxia and substrate insufficiency, which leads to the depletion of adenosine triphosphate (ATP), cellular swelling, and death. Vasoconstriction secondary to toxic or ischemic tubular epithelial injury further decreases glomerular filtration. It is important to note, however, that tubular lesions and dysfunction caused by toxic and ischemic insults can be reversible.

In contrast, the nephron damage associated with CKD is usually irreversible. Regardless of whether the underlying disease primarily affects the glomeruli, tubules, interstitium, or renal vasculature, irreversible damage to any portion of the nephron renders the entire nephron nonfunctional. Irreversibly damaged nephrons are replaced by fibrous connective tissue; therefore, a specific cause is rarely determined once end-stage kidney damage is present. CKD occurs

over a period of weeks, months, or years and is a leading cause of death in dogs and cats. Once advanced stage CKD has occurred, improving renal function is usually not possible. Conservative management of AKI includes fluid resuscitation, discontinuation and avoidance of nephrotoxic medications, nutritional support, correction of anuria or oliguria, symptoms control in terms of nausea and vomiting, and correction of electrolyte and acid-base imbalance (Lunn KF, 2011; Langston C., 2008).

A number of new treatments have recently emerged for AKI management in veterinary medicine, including dialysis techniques such as hemodialysis and peritoneal dialysis. However, they are often limited to few centers due to the need for special equipment and trained personnel (Segev et all., 2013 ; Langston et all., 1997) CDK is often regarded a result of repeated, small insults to the kidney (Nenov et al., 2000). However, one major injury may also lead to development of CKD (Venkatachalam et al., 2010). Consequently, AKI may result in CKD, but is also a complication thereof, since an individual with CKD can be predisposed to further injury, “acute-on-chronic disease” (Venkatachalam et al., 2010). Recently, an even closer interrelation between AKI and CKD has been proposed in both human and veterinary medicine (Cowgill et al., 2016; Chawla & Kimmel, 2012). It has been suggested that mechanisms of pathogenesis can be shared between the two. If this is true, CKD can be thought upon as a slow progressing acute, or ongoing, kidney injury (Cowgill et al., 2016).

1.1.3 CKD in dogs

Chronic Kidney Disease (CKD) is the most commonly diagnosed form of nephropathy in dogs and cats. It affects animals of all ages, but particularly affects elderly patients, with prevalence by 10% in dogs and 15% in cats over 15 years of age (Polzin, 2011). CKD is described as an irreversible and progressive disease, with a course that is typically not rapid, but prolonged for months or even years (Jacob et al., 2002).

CDK can be divided into glomerular or tubulointerstitial disease. In glomerular disease, dysfunction of the filtration barrier is present. Thus, the hallmark of glomerular disease is renal protein loss (renal proteinuria). Glomerular damage may be categorized as immune-complex glomerulonephritis (ICGN) or non-ICGN (amyloidosis, focal segmental glomerulosclerosis) (Cianciolo et al., 2016). Multiple genetic abnormalities that result in glomerular dysfunction have also been described (Littman et al., 2013; Nowend et al., 2012; Davidson et al., 2007). Tubulointerstitial disease refers to a disease process present in any area of the kidney apart from the glomerulus and pelvis, and examples that occur in dogs are renal dysplasia and Fanconi syndrome (Hoppe & Karlstam, 2000; Bovee et al., 1978). Chronic tubulointerstitial fibrosis is also the final pathway of CKD irrespective of underlying pathology, and the degree of tubulointerstitial damage is the morphological feature that is most closely associated with GFR (Nath, 1992; Schainuck et al., 1970).

Many different underlying mechanisms of renal damage may lead to CKD. These include inflammatory, immune mediated, infectious, vascular, metabolic or neoplastic disease, toxicity, trauma, and genetic predisposition. The exact aetiology is often unknown in dogs with a clinical diagnosis of kidney disease. The cause of CKD is usually difficult to determine. Because of the interdependence of the vascular and tubular components of the nephron, the end-point of irreversible glomerular or tubular damage is the same. A morphologic heterogeneity among nephrons exists in the chronically ill kidney, with changes ranging from severe atrophy and fibrous connective tissue replacement to marked hypertrophy. The histopathologic changes are not process-specific, and therefore the cause is usually unknown. Nevertheless, recent studies have shown that primary glomerular disorders are a major cause of CKD in the dog. Because glomerular filtration in toto is uniformly reduced, CKD can be considered a single pathologic entity, although many different pathways can lead to this end-point. Potential causes of CKD are listed in Table 1 (nelson couto, 2009).

POTENTIAL CAUSES OF CHRONIC KIDNEY DISEASE IN DOGS

Immunologic Disorders

- systemic lupus erythematosus
- Glomerulonephritis
- Vasculitis

Amyloidosis

Neoplasia

- Primary
- Secondary

Nephrotoxicants

Renal ischemia

Inflammatory or infectious Causes

- Pyelonephritis
- Leptospirosis
- Renal Calculi

Hereditary and Congenital Disorders

- Renal Hypoplasia or Dysplasia
- Polycyatic Kidneys
- Familial nephropathies

Urinary Outflow Obstruction

Idiopathic

Table 1 Potential causes of CKD

Etiology of CDK

The pathophysiology of CKD can be considered at both organ and systemic level. At the level of the kidney, the fundamental pathologic change that occurs is a loss of nephrons and decreased glomerular filtration rate (GFR). Reduced

GFR, in turn, results in increased plasma concentrations of substances that are normally eliminated from the body by renal excretion. Many substances have been shown to accumulate in the plasma of patients with CKD. The constellation of clinical signs known as the uremic syndrome is thought to occur, at least in part, as a result of increasing plasma concentrations of these substances. Components of the uremic syndrome include sodium and water imbalance, anemia, carbohydrate intolerance, neurologic disturbances, gastrointestinal tract disturbances, osteodystrophy, immunologic incompetence, and metabolic acidosis.

CKD develops over a period of months or years, and its clinical signs are often relatively mild for the magnitude of the azotemia. Unique signs of CKD include a history of weight loss, polydipsia-polyuria, poor body condition, nonregenerative anemia, and small and irregularly shaped kidneys. A diagnosis of CKD is usually based on a combination of compatible historical, physical examination, and clinicopathologic findings. Plain radiographs can confirm the presence of small kidneys. Renal ultrasonography will usually show diffusely hyperechoic renal cortices with loss of the normal corticomedullary boundary. The increased cortical echogenicity results from replacement of the irreversibly damaged nephrons with fibrous connective tissue. Radiographic studies and ultrasonography can also help identify or rule out potentially treatable causes of CKD, such as pyelonephritis and renal urolithiasis (Nelson Couto, 2009).

During the clinical course of progressive CKD when nephrons are continuously lost, remaining nephrons undergo hypertrophy. Single nephron GFR increases (glomerular hyperfiltration) because of afferent (and to a lesser extent, efferent) arteriolar relaxation (Brown et al., 1990; Deen et al., 1974). These adaptive responses can be considered beneficial in maintaining global GFR at first, but over time, glomerular hypertrophy and hyperfiltration are thought to contribute to glomerulosclerosis and further nephron loss (Finco et al., 1999; Brown et al., 1990; Brenner et al., 1982). At what level of decrease in nephron mass the inherent, self-perpetuating progression starts is unknown. Approximately 31/32 removals of renal mass in dogs resulted in moderate azotaemia (≈177-253 µmol/L) after allowing time for compensatory hypertrophy and hyperfiltration (Finco et al., 1999; Finco et al., 1994; White et al., 1991). This degree of azotaemia is common in dogs clinically diagnosed with CKD. Consequently, it has been suggested that self- perpetuating disease can be present in many dogs at diagnosis (Finco et al., 1999). Several clinicopathological variables have been associated with progression in dogs and people (Fig 4). Proteinuria is probably the variable with most evidence of an association with progression in dogs as well as in cats and people (Zoja et al., 2015; Chakrabarti et al., 2012; Li et al., 2010; Syme et al., 2006; Jacob et al., 2005; Peterson et al., 1995). The exact role of proteinuria in the pathogenesis and progression of canine CKD is uncertain, but recent evidence in canine medicine suggests that proteinuria can be a cause of tubulointerstitial damage rather than only a consequence of

glomerular or tubular dysfunction, as shown in people (Benali et al., 2013; Vilafranca et al., 1995; Eddy & Michael, 1988). Excess filtered protein results in increased proximal tubular cell pinocytosis of proteins, which in turn may result in cellular damage because of swelling and rupture of lysosomes and increased production of pro-inflammatory mediators (Benali et al., 2013; Vilafranca et al., 1995; Bertani et al., 1986). Proteinaceous casts can also obstruct the tubuli and contribute to intrarenal damage. The exact etiology of CKD may influence rate of progression in dogs and people, as may superimposed clinical or subclinical AKI, "acute-on-chronic disease" (Venkatachalam et al., 2015; Polichnowski et al., 2014; Williams et al., 1988). Other factors associated with rate of progression are systemic and glomerular hypertension (Lash et al., 2009; Finco, 2004; Jacob et al., 2003; Tozawa et al., 2003), intrarenal hypoxia (Tanaka et al., 2014; Mimura & Nangaku, 2010) and the mineral and bone disease associated with CKD, CKD-MBD (Lippi et al., 2014; Natoli et al., 2013). Recently, dehydration and osmotic stress were recognized as potential driving forces of progression in people with CKD (Gil et al., 2018; Clark et al., 2016). Reactive oxygen species generation, or "oxidative stress", may play a central part in the pathogenesis of progression of CKD of both dogs and people, potentially involving several of the aforementioned factors (Kogika et al., 2015; Xu et al., 2015; Brown, 2008).

Parenchymal destruction and fibrosis is the final pathway in the pathogenesis and the common end-point of progressive CKD, regardless of aetiology.

Fibrosis, which may affect all parts of the kidney, is called glomerulosclerosis when affecting the glomerular compartment, and tubulointerstitial fibrosis when affecting the tubulointerstitial compartment. Because of the intimate connections between these functional parts, disease in one compartment negatively affects the other. An example of this is the tubulointerstitial damage that may arise secondary to glomerular proteinuria (Lazzara & Deen, 2007). The division of renal fibrosis pathophysiology into four phases has been suggested, based on studies predominantly in rats and mice (Eddy, 2000). Recent studies have shown similar histopathologic patterns in canine progressive CKD (Benali et al., 2014; Aresu et al., 2011). The first phase, "cellular activation and injury", is characterized by recruitment of inflammatory cells and appearance of myofibroblasts. In the second "fibrogenic signalling" phase, the number of myofibroblasts increase. During the third, "fibrogenic" phase, accumulation of ECM occurs because of an imbalance between matrix synthesis and degradation. In the fourth, "renal destruction" phase, ECM accumulation and nephron destruction is seen. The start of the fourth phase was described as the point of irreversibility, because of permanent destruction of renal structural elements. These phases have been studied in animal models after injury at one point in time, but in spontaneous disease many or all of these phases may be observed histologically at the same time (Eddy, 2000). Synthesis, degradation and renal accumulation of ECM proteins and induction of proteases and other ECM-remodelling enzymes all contribute

to development of renal interstitial fibrosis and disease progression (Eddy, 2014).

Renal tubular epithelial cells are thought to play a role in both the initial lymphocyte recruitment and in the fibrogenic phases as progenitors of the increasing population of myofibroblasts in the canine kidney (epithelial to mesenchymal transition (Benali et al., 2014). The induction and proliferation of myofibroblasts is thought to represent a central event in the initiation and propagation of fibrosis in dogs and people (Benali et al., 2014; Genovese et al., 2014).

CKD is generally classified into four stages based on laboratory tests and clinical signs. In Stage 1, a process is damaging the kidneys but azotemia and clinical signs have not developed (Creatinine<1.4 mg/dl). Unfortunately, renal disease is uncommonly detected at this stage. In Stage 2, the disease has progressed, GFR has fallen to <25% of normal, and azotemia is present, but clinical signs are not yet seen (creatinine 1.4<>2 mg/dl). However, this stage may be associated with impaired urine-concentrating ability and increased urine volume. Stage 3 occurs when GFR has declined further, azotemia is present, and clinical signs are often seen (creatinine 2.1<>5 mg/dl). Stage 4 reflects further progression and severe azotemia, with clinical signs present. This staging system applies to CKD only (Table 2)

SERUM CREATININE CONCENTRATION	STAGE I NONAZOTEMIC CKD	STAGE II MILD RENAL AZOTEMIA	STAGE III MODERATE RENAL AZOTEMIA	STAGE IV SEVERE RENAL AZOTEMIA
mg/dl (cats)	<1.6	1.6-2.8	2.9-5.0	>5.0
mg/dl (dogs)	<1.4	1.4-2.0	2.1-5.0	>5.0

IRIS, International Renal Interest Society; CKD, chronic kidney disease.

Table 2 2IRIS CKD Staging System for Dogs and Cats

Attempting to identify the primary process causing the kidney disease, especially in Stages 1 and 2, is important to form a prognosis and treatment plan. Known causes of CKD include diseases of the macrovascular compartment (systemic hypertension, coagulopathies, chronic hypoperfusion), microvascular compartment (systemic and glomerular hypertension, glomerulonephritis, developmental disorders, congenital collagen defects, amyloidosis), interstitial compartment (pyelonephritis, neoplasia, obstructive uropathy, allergic and immune-mediated nephritis), and tubular compartment (tubular reabsorptive defects, chronic low-grade nephrotoxicity, obstructive uropathy). Many causes of chronic, generalized renal disease are associated with progressive interstitial fibrosis. The severity of interstitial fibrosis is positively correlated with the magnitude of decline of GFR and negatively correlated with the prognosis. The glomerular, tubulointerstitial, and vascular lesions found in animals with generalized CKD are often similar, regardless of the initiating cause, particularly in Stage 4. At this point, renal histology shows

only marked interstitial fibrosis, called chronic interstitial nephritis or tubulointerstitial fibrosis. This term describes the morphologic appearance of kidneys with end-stage chronic disease of any cause. Because AKI may progress to a chronic condition, any cause of acute kidney injury is also a possible cause of CKD. Generally, no clinical signs are seen as a direct result of disease until ≥75% of nephron function has been impaired (Stages 3 and 4). The earliest clinical signs commonly attributable to renal dysfunction are polydipsia and polyuria, which are not seen until the function of approximately two-thirds of the nephrons has been impaired (late Stage 2 or early Stage 3). Further destruction of renal tissue leads to azotemia without new clinical signs in Stage 2, and finally to the clinically apparent uremic syndrome in Stage 4. Initially, uremia is associated with occasional vomiting and lethargy. As the disease progresses within Stages 3 and 4 throughout months, anorexia, weight loss, dehydration, oral ulceration, vomiting, and diarrhea become fully manifest. Physical examination and imaging studies of animals in Stages 3 and 4 usually reveal small, irregular kidneys, although normal to large kidneys can be seen in animals with neoplasms, hydronephrosis, or glomerulonephritis. Mucous membranes are pale in late Stage 3 and Stage 4, due to the presence of a nonregenerative, normocytic, normochromic anemia.

Serum creatinine concentrations should always be interpreted in light of the patient's urine specific gravity and physical examination findings to rule out

prerenal and postrenal causes of azotemia. The CKD stages are further classified by the presence or absence of proteinuria and systemic hypertension. The classic diagnosis of renal failure based on renal azotemia (persistent azotemia superimposed on the inability to concentrate urine) pertains to CKD stages 2 through 4. Stage 1 CKD (nonazotemic CKD) could be diagnosed in dogs with persistent proteinuria, urine-concentrating deficits, increases in serum creatinine concentration over time even if the values remain in the normal range (serum creatinine concentration that increases form 0.6 to 1.2 mg/dl could indicate a 50% reduction in GFR), or abnormal renal palpation or renal ultrasonographic findings.

Clinical Signs and Therapy

Beckel et al., (2005) reported clinical signs at presentation included lethargy (100%), vomiting (100%), anorexia (60%), diarrhoea (20%), polyuria/polydipsia (20%), and hind limb stiffness (20%) in ARF due to leptospirosis. Robertson and Seguin, (2006) opined that many cases of chronic renal failure are asymptomatic (other than PU/PD) until dehydration leads to decompression, leading to more acute history from the owner perspective. Grauer, (2009) stated that decreased production of erythropoietin contributes to the non-regenerative anaemia of CKD and decreased metabolism and excretion of parathyroid hormone and gastrin contribute to osteodystrophy and

gastritis respectively. Geigy et al., (2011) reported systolic systemic hypertension (>160 mmHg) and severe systolic systemic hypertension (>180 mmHg) as 37% and 15% at admission and increased with hospitalization to 81% and 62%, respectively in a study on occurrence of systemic hypertension in dogs with acute kidney injury. Tufani et al., (2015) reported gastrointestinal abnormalities were the commonest clinical outcome of renal disease in dogs, which included chronic vomiting (84% cases), halitosis (68% cases), oral lesions/ulceration (54% cases), loss of appetite (89% cases),polyuria/polydipsia (52.38% in CRF and 20.69% in AKI), oliguria/anuria (79.31% in AKI, 47.62% in CRF and 100% in obstructive urolithiasis) and haematuria (55.17% in AKI,28.57% in CRF). Anaemia was common manifestation of both acute (77.59%) and chronic (88.10%) renal failure. With appropriate therapy, animals can survive for long periods with only a small fraction of functional renal tissue, perhaps 5%–8% in dogs and cats. Recommended treatment varies with the stage of the disease. In Stages 1 and 2, animals usually have minimal clinical abnormalities. Efforts to identify and treat the primary cause of the disease should be thorough. The systemic hypertension seen in ~20% of animals with CKD may be seen at any stage and is not effectively controlled by feeding a low-salt diet. If an Angiotensin-Converting Enzyme (ACE) inhibitoris used in conjunction with a renal diet, potassium should be carefully monitored. Hyperkalemia may develop, particularly in Stage 4, and dietary change or dosage adjustment should be

considered if serum potassium exceeds 6.5 mEq/L. ACE inhibitors and calcium-channel blockers may be administered together, an ACE inhibitor is usually recommended as initial therapy in dogs. In addition to providing a continuous supply of fresh drinking water and encouraging adequate dietary intake, body condition scoring should be used routinely to assess adequacy of intake. Animals in this stage should be fed standard, commercially available maintenance diets, unless they are markedly proteinuric. All affected animals should be checked every 6–12 mo, or sooner if problems develop. In Stages 2 and 3, the principles for management of complications are the same, except that the animal should be evaluated every 3–6 mo. These evaluations should include hematology, serum biochemistries, and urinalysis. Because dogs with CKD are prone to development of bacterial urinary tract infections, urine culture should be performed annually, and any time urinalysis suggests infection. The progressive nature of this disease produces a vicious cycle of progressive renal destruction. Measures that may slow this progression include dietary phosphorus restriction, dietary fish oil supplementation, antihypertensive agents (for hypertensive dogs), and administration of ACE inhibitors. Dietary restriction of phosphate and acid load is essential in this stage, and specialized diets for management of kidney disease should be fed. There is also a clear rationale for the inclusion of dietary n-3 polyunsaturated fatty acids in these stages. In late Stage 3 and Stage 4, all of the principles of managing the preceding stages apply, except that the animal should be evaluated every 1–3

mo. Dietary restriction of protein may relieve some of the signs of uremia. Administration of a proton pump inhibitor such as omeprazole decreases gastric acidity and vomiting. Fluid therapy with polyionic solutions, given IV or SC in the hospital or SC by owners at home, is often beneficial in animals with intermittent signs of uremia. Oral vitamin D administration may reduce uremic signs and prolong survival, particularly in dogs. Probiotic medications and certain dietary fibers may enhance gut catabolism of nitrogenous compounds and uremic toxins.

Prognosis

The long-term prognosis for dogs with a diagnosis of CKD is often poor. However, the outcome is considerably variable between dogs, partly because of differences in progression rates and partly as a result of the definition of canine CKD. Some dogs that are given a diagnosis of CKD at an early stage, do not develop progressive CKD. Also, a CKD diagnosis can be based on persistent (>3 months) renal proteinuria, which may later resolve. Consequently, canine CKD, as currently defined in veterinary medicine, may be either static (non-progressive) or active (progressive). When the disease is progressive, most dogs proceed to end-stage disease and death, but rate of progression is variable both within and between individuals (Finco et al., 1999). It is difficult to predict the rate of progression in individual dogs. The fact that

progression presumably is neither linear nor predictable is also recognized in human medicine (Onuigbo & Agbasi, 2014).

1.1.4 Management and Prevention of CDK

Dehydration

Michell et al., (1989) stated that a rough estimation of volume replacement may be made from the PCV alone. A common formula, assuming the extracellular deficit was to allow 10ml/kg body weight for each 1% rise in PCV above normal level. Adams, (2004) stated that chronic administration of subcutaneous balanced electrolyte solution has been advocated to prevent dehydration, maintain renal blood flow and GFR, increase urine output and ameliorate clinical manifestation of uraemia. Maddison and Syme, (2010) stated that fluid therapy is an essential component of management of CKD and fluid rate should be approximately twice normal of the maintenance rates. The fluid required for maintenance is approximately equals to 50/ml/kg. Yozova et al., (2016) studied retrospective evaluation of the effects of administration of tetrastarch (hydroxyethyl starch) on plasma creatinine concentration in dogs and concluded – HES administration in this canine population under investigation did not result in increased creatinine concentrations compared to administration of crystalloids. However, recent large clinical trials and meta-analyses showed

an increased incidence of acute kidney injury (AKI) and mortality in critically ill and septic human patients receiving HES products due to their side effects such as coagulopathies, kidney injury and tissue storage. As a result, HES products are no longer recommended in critically ill, septic or burn patients, as well as patients with renal impairment or coagulopathies.

Management of anaemia

Cowgill et al., (1998) conducted study to test efficacy and safety of recombinant human erythropoietin (r-HuEPO) administration in dogs and cats with naturally developing chronic renal failure. They concluded administration of r-HuEPO has the potential to resolve anaemia and improve clinical well-being. Macdougall and Cooper, (2002) conducted study on the role of inflammation and pro□inflammatory cytokines in erythropoietin resistance and observed that chronic renal failure shares several features of the inflammatory state like elevated circulating levels of inflammatory cytokines such as interleukin-6 which may be associated with poor response to erythropoietin treatment in end stage renal disease. So, strategies utilizing anti-cytokine therapy may prove to be a useful adjuvant in optimizing the response of r-HuEPO therapy.

Randolph et al., (2004) reported significant improvement in packed cell volume (PCV), red blood cell count and haemoglobin concentration after 1 week of

treatment with erythropoietin. They did not observe immunogenicity problems with rHuEPO administration to dogs with use of rcEPO. But reported rcEPO was not as effective in restoring erythrocyte production in dogs that had previously developed rhEPO-induced red cell aplasia. Sukullaya and Anuchai, (2008) in a retrospective study of clinical use of recombinant human erythropoietin for treatment of anaemia in dogs with renal failure and concluded treatment with r-HuEPO stimulated erythrocyte production in dogs with naturally developing anaemia of chronic renal failure during 8-week treatment period. Exogenous r-HuEPO had no effect on leukocyte, platelet counts and serum biochemistry in these uremic dogs.

Management of gastrointestinal signs of uraemia

Perkovic et al., (2002) reported 5-HT3 receptor antagonist ondansetron to be twice as effective as metoclopramide in reducing uremic nausea and vomiting in the symptomatic relief of uraemia-induced nausea and vomiting. Polzin, (2011) stated that the presence of gastrointestinal complications of CRF is sufficient reason to warrant reducing dietary protein intake.

Management of hyperkalemia

Rang et al., (2007) reported that the use of potassium sparing diuretic, spironolactone to prevent potassium loss in cases with hypokalemia which could be caused by administration of furosemide.

Lehnhardt and Kemper, (2011) reviewed on management of severe hyperkalemia due to acute and chronic renal failure and stated that hyperkalemia can be successfully managed by using dextrose or insulin infusion, Beta-adrenergic agonists (salbutamol, reproterol) which stimulate potassium to shift from extra to intracellular space via Na+/K+-ATPase, Sodium bicarbonate and increased potassium excretion by using loop diuretics (furosemide) which inhibit the inward transport of potassium.

Management of hypertension and proteinuria

Uzlu and Kalnbacak, (2005) reported that birbesartan, an angiotensin II receptor antagonist, was effective and safe in treatment of CRF associated hypertension.

Lefebvre et al., (2007) reported the use of ACE-inhibitors i.e. enalpril in dogs with CRF as in reduced glomerular capillary pressure, had antiproteinuric effect, tended to delay the progression of CRF and limit the extent of lesions.

Tenhündfeld et al., (2009) conducted clinical trial of 26 dogs with spontaneous CKD evaluated effects of the ACEI benazepril alone, benazepril plus short-term heparin therapy and placebo in a 6-month clinical study and suggested that administration of benazepril had beneficial effects in dogs with chronic kidney disease but that short-term administration of heparin in conjunction with benazepril did not appear to provide any additional benefit.

A study by Nakamoto H. in 1994 in dogs demonstrated the important contribution of L-arginine, administered orally, in modulating the increased activity of the renin-angiotensin system during evolution of renal vascular hypertension causing renal dysfunction.

Management of hyperphosphatemia

Polzin et al., (2000) advocated the use of sucralfate for the gastrointestinal ulceration and to bind the phosphorus in the intestine of the dogs with chronic renal failure.

Polzin et al., (2005) studied clinical benefit of calcitriol in canine chronic kidney disease and concluded dogs with azotemic CKD that were treated with calcitriol therapy reduces mortality in dogs with CKD and reported median of 365 days survival as compared to 250 days in dogs treated with placebo.

Cozzolino et al., (2014) studied preclinical pharmacokinetics, pharmacodynamics and safety of sucroferric oxyhydroxide. Sucroferric

oxyhydroxide is a polynuclear iron-based phosphate binder recently approved for the treatment of hyperphosphataemia in patients with CKD and concluded sucroferric oxyhydroxide offers a new option for the treatment of hyperphosphataemia, with a high phosphate binding capacity, minimal iron release and low potential for iron accumulation and toxicity.

Dietary management

Because hyporexia and anorexia and their associated adverse nutritional effects progressively lead to a devastating deterioration in the patient's quality of life, it is essential that they be recognized and responded to appropriately early in the course of CKD. The signpost that should be sought is whether the patient is meeting the nutritional goals of a stable body weight at an acceptable nutritional status (measured as a body condition score and assessment of activity and strength). There is strong evidence supporting a recommendation to feed a renal diet to dogs and cats with serum creatinine concentrations in excess of 2.0 mg/dl (176 µmol/l; CKD Stages 3 and 4 in dogs and mid-2 through 4 in cats) (Polzin, 2009). Benefits shown to accrue from dietary therapy in dogs and cats include preventing or delaying the onset of uremia and premature death due to complications of CKD. At least in dogs, these benefits have been shown to accrue, at least in part, from slowing progression of CKD. Importantly, in these diets have been shown to maintain or improve nutrition compared to

consumption of a maintenance type diet. A common misconception is that renal diets are simply "low protein diets." Renal diets encompass a variety of modifications beyond just a limitation of protein content, and, indeed, the principal beneficial effects of these diets may not accrue from their protein content. Thus, simply replacing a renal diet with a standard manufactured diet that is lower in protein content does not meet the guideline of feeding a renal diet. Since inappropriate diets can exacerbate clinical signs of uremia and/or promote progression of CKD, cats and dogs with CKD should be fed a renal diet.

Owners often consider food consumption to be a premier indicator of their pet's quality of life, and they are often happy when their pet shows any interest in food. However, it is inappropriate to accept the pet's consumption of "some" food as a goal of therapy. Malnutrition is a major cause for morbidity and mortality in dogs and cats with CKD stages 3 and 4 (Polzin, 2009).

Elliott, (2006) stated that the dogs with mild to moderate CRF fed a renal diet experienced a 70% reduction in the relative risk for developing a uremic crisis, remained free of uremic signs almost 2.5 times longer and had a median survival that was three times longer than those with CRF fed a maintenance diet. Renal function declined more slowly in the dogs fed the renal diet.

Yu et al., (2006) studied effects of dietary supplementation of a dry therapeutic renal food with antioxidants (vitamins E and C, and carotenoids) in dogs suffering from CKD and reported compared with baseline, antioxidant

supplementation significantly reduced oxidative stress and serum creatinine concentration compared with dogs receiving the renal food without antioxidants.

Polzin (2010) advocated that renal diets have been shown to improve nutrition, higher quality of life scores than with maintenance diets.

Peritoneal dialysis

Beckel et al., (2005) reported 90% success rate of peritoneal dialysis in the management of dogs with acute renal failure (ARF) caused by leptospirosis. Dorval and Boysen, (2009) conducted retrospective study on management of acute renal failure in cats non-responsive to medical therapy using peritoneal dialysis andconcluded PD as an effective renal replacement therapy for ARF in cats and carry a reasonable prognosis in selected case with 83% success rate.

Vitalaru and Micsa, (2015) reported the successful treatment of acute renal and liver failure in cat using peritoneal dialysis and found it as an effective method to resolve elevated levels of creatinine and urea, hyperkalemia, hyperphosphatemia and metabolic acidosis which do not yield to treatment can be solved using peritoneal dialysis. It also has a good effect in acute liver failure, cleaning the high levels of bilirubin.

1.2 The cardiovascular system

The cardiovascular system consists of the heart and blood vessels, namely arteries, veins and capillaries and its primary function is circulation of blood throughout the body. Blood vessels form a conduit system throughout the body and carry blood to all organs, tissues and cells.

1.2.1 Anatomy of the heart

The heart is located in the chest between the right and left lungs and is contained in a very thin sac called the pericardial sac and its contractile tissue is called myocardium. The heart is positioned in the thorax surrounded by a fibrous sac, the pericardium. The external layer of the heart tissue is called the epicardium and the innermost layer in connection to the ventricles the endocardium. The tissue between the two aforementioned layers, the myocardium, is responsible for ventricular contraction and consists of muscular tissue. The heart is divided into a left and a right side by the septal wall as illustrated in Figure 3.

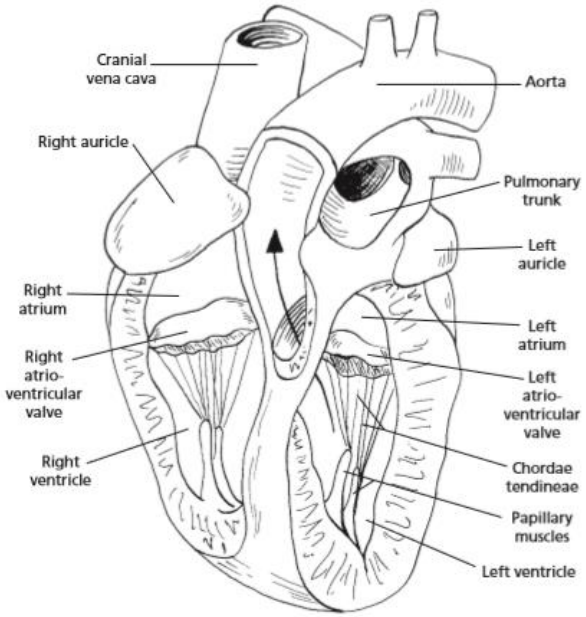


Figure 3Tthe anatomy of the canine heart

Each side of the heart consists of two chambers, the atrium and the ventricle, separated by an atrioventricular (AV) valve: the mitral and the tricuspid valves on the left and right sides, respectively. The left side of the heart delivers oxygen-rich blood to the body (systemic circulation) passing through the aortic valve to the aorta, whereas the right side pumps blood through the pulmonary valve and the pulmonary artery for an oxygen refill in the lungs (pulmonary circulation).

The four heart valves act as inlet and outlet check-valves for the ventricles, allowing unidirectional flow and preventing backflow by being passively opened and closed due to pressure gradients. The plane that separates the

ventricles from the atria is often referred to as the AV-plane, where all the valves of the heart are situated. Returning blood from the body flows to the right atrium through the inferior and superior vena cava, while blood from the lungs returns to the left atria through the pulmonary vein. The tip or the pointed end of the heart is called the apex, and the region opposite the apex is known as the base of the heart. The heart extends approximately from the 3rd to the 6th rib of the dog. The heart is the central organ that contracts rhythmically to pump blood continuously through the blood vessels. The heart consists of four chambers, two atrium (right and left) and two ventricles (right and left). The right atrium is the collecting chamber for blood from distant parts of the body. As the right atrium contracts, blood flows through the tricuspid valve into the right ventricle. Right ventricle contracts and sends blood which it has received from the right atrium into the pulmonary artery for oxygenation. Left atrium receives the oxygenated blood from pulmonary vein and send into left ventricles through mitral valve which pumps blood in systemic circulation completing the one cycle of cardiac event (Figure 4).

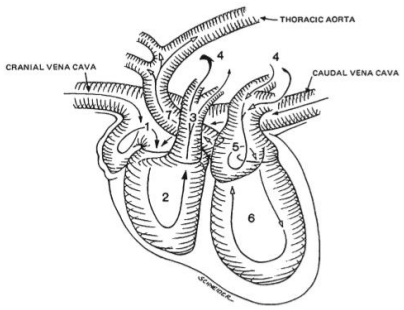


Figure 4 Schematic of the blood pathway through the heart

The circulatory system transports oxygen, nutritive substances, immune substances, hormones and chemicals necessary for normal function and activities of the tissues and organs of the body. It also carries away waste products and carbon dioxide, helps to regulate body temperature, and helps to maintain normal water and electrolyte balance. Heart being one the vital organ of the body which supplies the oxygenated blood to almost every tissue and being responsible for maintaining homeostasis and optimum functioning of different body systems.

1.2.2 The cardiac cycle

The right and the left sides of the heart operate as two serial pumps, performing the pumping work fairly synchronously in the normal heart. The cardiac cycle consists of the two main phases: systole, referring to the period of ventricular contraction and ejection of blood out of the ventricles, and diastole, being the period of ventricular relaxation and filling. The myocardial contraction is initiated by spontaneous generation of an electrical impulse in the sinus node located in the superior lateral wall of the right atrium. The contraction results in a pressure rise within the ventricles. The phase when pressure increases most rapidly is called the isovolumic contraction time (IVCT), since all valves in the heart are closed and the ventricular volume is constant. When the pressures in the ventricles exceed the pressures in the aorta and the pulmonary artery, the

aortic and pulmonary valves open and the ventricles eject blood. The blood travels along the cardiovascular system, driven by the pressure gradient generated by the myocardium. Blood from the body and the lungs starts to fill the atria of the heart, simultaneously with ventricular ejection. The pulmonary and aortic valves close when the pressures in the aorta and the pulmonary artery exceed the ventricular pressures. When the ventricular myocardium starts to relax, the ventricular pressures drop rapidly. Once again, all valves in the heart are closed during the isovolumic relaxation time (IVRT). The pressures in the blood-filled atria increase, which leads to mitral and tricuspid valve opening to let the blood flow into the ventricles. During the first part of the ventricular filling phase, the blood flows as a result of the pressure gradient between the atria and the ventricles. This phase is called the fast filling phase or the early diastolic wave (E-wave). The filling continues after the E-wave, but at a reduced rate during the phase called the diastasis. This phase is followed by atrial contraction during the atrial diastolic wave (A-wave), contributing to the ventricular filling by lifting the AV-plane. The pressures inside the ventricles rise with the increased filling and, finally, the blood is pushed up against the mitral and tricuspid valves, forcing them to shut. Thereafter a new cardiac cycle can begin. Myocardial perfusion mainly occurs during diastole, as a consequence of increasing resistance in the coronary arteries during systole.

1.2.3 Vascular anatomy and function

The blood vessels provide a continuous route for blood leaving the heart to return to the heart. From the ventricles back to the atria they are, in order, the arteries, arterioles, capillaries, venules, and veins.

All vessels, except for the exchange vessels, the capillaries, have the same basic structure. Only the composition of the layers, and the fraction of different cells in the layers, are dependent on vessel type. The vessel wall is arranged in three layers; tunica intima, tunica media and tunica adventitia. The tunica intima is the innermost layer, which is in direct contact with the blood. This layer consists of endothelial cells surrounded by a thin layer of connective tissue, providing the vessel with a smooth inner surface. The tunica media is the middle layer of the vessel wall, consisting of smooth muscle and elastic and collagen fibers in helically arranged medial layers. Tunica media is the principal determinant of the mechanical properties of the arteries. The media is connected to the intima and adventitia with elastic membranes, the internal and external elastic lamina. The vessel is covered by loose connective tissue in its third layer, the adventitia. This layer consists of thick bundles of collagen fibrils arranged in helical structures which stabilize and strengthen the vessel. In larger arteries, this layer also contains a network of small blood vessels supplying the vessel, the vasa vasorum. The elastic arteries are the largest arteries, i.e. the pulmonary artery, the aorta and their major branches. They are termed elastic

arteries because elastic fibers are dominant in their vessel walls. The function of the elastic arteries is to transform the accumulated potential energy from the ejection phase into kinetic energy during diastole, in order to keep up a more continuous flow in the arteries. The walls of an elastic artery easily expand and recoil. Arterial compliance is defined as the change in arterial volume divided by the associated distending pressure. Muscular arteries are located at more peripheral parts of the circulatory system and their main function is to distribute blood to different parts of the body. They are called muscular arteries, since they contain more smooth muscles and less elastic tissue than the elastic arteries. Small arteries and arterioles are termed resistance vessels, since they account for the greatest part of the resistance in the vasculature. The arterioles distribute and regulate the blood flow to the capillaries by adjusting the vessel diameter and thereby the resistance in the vessels. The smallest vessels in the cardiovascular system are the capillaries and their function is to exchange nutrients and gases between the blood in the capillaries and the surrounding tissue. The capacitance vessels, the venules and veins collect and lead the blood back to the heart again. They work as a blood reservoir and thus can control the returning blood to the heart.

The largest vein in the animal's body is the vena cava, and all other systemic veins eventually drain into it. Many veins are working against gravity to get the blood back to the heart and they don't have the force of ventricular contraction to propel blood flow. For this reason small and medium veins have

one-way valves in their lumens. The valves allow blood to flow only in the direction of the heart. When blood tries to flow backward, the valves close. These valves are similar to the semilunar valves in the heart, but they each have only two cusps. Muscular movements in the body compress small veins and the one-way valves allow blood to move only toward the heart. This is the only mechanism that propels blood back to the heart (Figure 5).

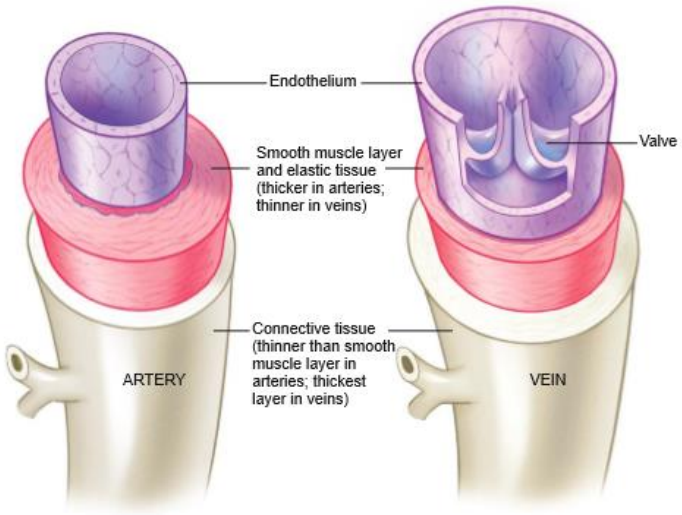


Figure 5 Anatomy of Arteries and veins

1.2.4 Cardiac Diseases

Variety of infectious, non-infectious, genetic, nutritional, environmental, and parasitic diseases can adversely affect the heart leading to its compromised function and may act as potential cause of death losses to canine population.

Egenvall et al. (2006) found that the cardiac-related mortality for dogs <10 years of age, was 21.3 deaths per 10,000 dog years at risk (DYAR). This mortality in males and females was 27.3 deaths and 15.4 deaths per 10,000 DYAR, respectively. The 3 breeds with the highest point estimates were Irish Wolfhounds, Cavalier King Charles Spaniels, and Great Danes (356, 247, and 179 deaths per 10,000 DYAR) respectively. According to information disseminated by the American Veterinary Medical Association, one in ten dogs suffers from heart disease (Dove, 2001). While some animals have a genetic predisposition toward the development of heart disease, other many develop degenerative heart conditions as a result of multiple dietary factors, including nutritional deficiencies, obesity, or cachexia. Hence any cardiac abnormality requires to dealt with on priority to avoid morbidity and mortality.

The most commonly encountered cardiac diseases of dogs are hypertension, ischemic heart diseases, congestive cardiac failure, valvular diseases, aortic aneurysm and peripheral arterial diseases. Heart diseases in dogs are either acquired or congenital. The overall prevalence of cardiac diseases in the dog is around 4.4% (Manczur et al., 2003). Congenital heart diseases are more prevalent in purebred dogs. The prevalence rate of congenital cardiac diseases has been reported to about 2.8% (Brambilla et al., 2003). The most commonly diagnosed congenital heart defects in the dog includes aortic stenosis, patent ductus arteriosus (PDA), pulmonic stenosis, ventricular septal defects, mitral valve dysplasia, tetralogy of Fallot and endocardial fibroelastosis (Ware, 2003).

A great deal of differences in the population of dog breed exits from region to region, which also affects the prevalence of cardiac diseases. However, the combined congenital defects are reported to be much less and account only to about 0.3% of total dogs affected with heart diseases (Brambilla et al., 2003). Acquired heart diseases which are more prevalent than congenital, includes dirofilariasis, mitral regurgitation (endocardiosis; MR), cardiomyopathies (dilated cardiomyopathy, hypertrophic cardiomyopathy), cardiac arrhythmia, hypertension, endocarditis, and pericardial effusion (Atkins, 2007).

Cardiomyopathy, or heart muscle disease, describes a group of heterogeneous conditions that affects the heart muscle functionally and/or structurally or morphologically. Primary cardiomyopathies are idiopathic, and no underlying disease can be identified. Secondary cardiomyopathies are a consequence of an underlying pathology (e.g. carnitine deficiency cardiomyopathy, doxorubicine cardiotoxicity, tachycardia-induced myocardial failure). Cardiomyopathy can be classified as Idiopathic and specific cardiomyopathies. The Idiopathic cardiomyopathy includes (Idiopathic Dilated cardiomyopathy (IDCM), Idiopathic Hypertrophic cardiomyopathy (IHCM), Idiopathic Restrictive cardiomyopathy (IRCM), and Idiopathic Arrhythmogenic right ventricular cardiomyopathy (ARVC). Specific cardiomyopathies consist of hypertensive cardiomyopathy, endocrine cardiomyopathy, metabolic cardiomyopathy and ischemic cardiomyopathy. Cardiomyopathy is primarily disease of myocardium of heart and characterized by dilation of all the cardiac chambers,

but in particular left ventricular chamber dilation is more commonly reported. Cardiomyopathy impairs the contractility of myocardium leading to mainly systolic dysfunction; however, diastolic dysfunctions have also been reported (Richardson et al., 1996; Tidholm and Jonsson, 2005). Dilated cardiomyopathy (DCM) is disease of unknown etiology characterized by dilatation of one or both ventricles with severe impairment of systolic function in absence of other detectable cardiovascular disorder. Metabolic disorders associated with DCM include hypothyroidism, diabetes mellitus, and pheochromocytoma (Atkins, 1991). However, immunologic disorders (auto-antibodies have been detected against several cardiac structures, such as the β-adrenergic receptor (Limas, 1996), mitochondria and the myosin heavy chains), inflammatory response to infectious agents, such as viruses (Cambridge et al., 1979; Muir et al., 1989), bacteria (Stanek et al., 1990) and protozoa (Barr, 1991) has been proposed to be involved in the pathogenesis of myocarditis and DCM. Cardiotoxicity leading to DCM may also be caused by doxorubicin and other antineoplastic agents, ethanol, cobalt, lead, catecholamines, histamine, methylxanthines, and vitamin D (Van Fleet and Ferrans, 1986).

Dilated cardiomyopathy

Dilated cardiomyopathy (DCM) is the most common myocardial disease of the dog. DCM is characterized by profound dilation and severely reduced

contractility of the left ventricle. The atria dilate secondarily due to increases in ventricular diastolic pressures. Thus, DCM is primarily a disorder of systolic function, although ventricular filling abnormalities may also be present. DCM is one of the most common acquired heart diseases in the dog. Canine DCM has been recognized in several breeds, notably boxer dogs, Dobermanns, English cocker spaniels, and in giant breed dogs (Tidholm, 1996). Boxers with dilated cardiomyopathy (historically known as Type III Boxer cardiomyopathy) were identified as having a myocardial L-carnitine deficiency (Keene et al., 1988; Keene et al., 1991). Similarly, American Cocker Spaniels have been reported to develop DCM associated with low taurine levels (Torres et al.,2003). DCM with secondary congestive heart failure, age of onset ranged from 3.5 to 13 years with a mean and median of 6.6 years. In contrast, Portuguese Water Dogs (PWDs) develop a form of cardiomyopathy that occurs much earlier (13±7.3 weeks) of life (Dambach et al., 1999).The most commonly observed symptoms of cardiomyopathy are exercise intolerance, dyspnoea, tachypnoea, and coughing (due to left sided heart failure); abdominal enlargements, jugular distension and pulsation (due to right sided heart failure), cold extremities, loss of weight, syncope, low- intensity systolic murmur, weak femoral arterial pulses, and cyanosis (Tidholm et al., 1997). Thorough anamnesis, physical, clinical, biochemical analysis along with electrocardiography, radiography and echocardiography could make it possible to diagnose the most of cardiac abnormalities. Hence all the dogs with these

symptoms requires careful monitoring. However, the cardiac diseases may be secondary to renal diseases (Valocikova et al., 2008) diabetes (Kamble and Vaidya, 2002), pulmonary diseases (Pyle et al., 2004) and air pollutions (Simkhovich et al., 2008) leading to multisystemic effects in cardiac patients.

Diagnosis of DCM is based on breed, clinical signs, physical examination, electrocardiography and thoracic imaging. On physical examination, a soft, systolic regurgitant murmur may be heard over the mitral or tricuspid valves. An early diastolic (S3) gallop associated with rapid inflow into a dilated ventricular chamber may also be auscultated. Other abnormalities include pale mucous membranes and slow capillary refill time, cardiac arrhythmias with pulse deficits, and hypokinetic pulses. Dogs with right-sided heart failure will have ascites, pleural effusions, and distended jugular veins. Premature ventricular contractions and ventricular tachycardia are commonly seen in Doberman Pinschers and Boxers with DCM, possibly predisposing them to sudden death. (Calvert C.A.,1992) Atrial fibrillation is a common rhythm disturbance seen in large- and giant-breed dogs with DCM. Other abnormal electrocardiographic findings include wide P waves, tall or wide R waves, and ST segment changes. Thoracic imaging, including thoracic radiographs and echocardiography, is essential for definitive diagnosis of DCM. Generalized cardiomegaly with signs of left- or right-sided heart failure is a typical radiographic feature of canine DCM. Dilated ventricles, an enlarged left atrium, increased E-point septal separation, and impaired systolic function are

echocardiographic features of DCM. The diagnosis of DCM in asymptomatic patients is difficult.

Pulmonary Hypertension

Pulmonary hypertension (PH) stems most often from chronic left heart failure, dirofilariasis, and severe interstitial lung diseases; PH also can be idiopathic (primary) in dogs. PH is very common in dogs with chronic mitral regurgitation (MR) and typically leads to signs of low cardiac output, a progressively louder murmur of tricuspid regurgitation, and signs of right sided failure (ascites and exertional syncope). Pericardial effusion is a frequent cause of heart failure in dogs but often is misdiagnosed. Right-sided CHF, including pleural effusion, can occur in chronic cardiac tamponade. Often cardiac related neoplasia is involved (hemangiosarcoma, chemodectoma, mesothelioma, ectopic thyroid neoplasia). Treatment does not involve drugs but pericardiocentesis often followed by surgical procedures. Cardiac arrhythmias often complicate these disorders and may precede development of heart failure. Tachyarrhythmias, if relentless (as with sustained atrial flutter or reentrant supraventricular tachycardia) induce a reversible decrease in ventricular function, additive to preexisting disease. Bradyarrhythmia such as sinus arrest and atrioventricular block are more often related to primary disease of the conduction system in

dogs. Cardiac arrhythmias are considered author elsewhere. Treatments involve drug therapy, pacing, or catheter interventions (Bonagura J.D., 2010).

Valvular Degeneration (Endocardiosis)

Degenerative valvular disease, particularly of the mitral valve, is the most common cardiovascular disease in the dog. This disease is primarily seen in older, small-breed dogs; 30% to 35% of dogs over 13 years of age exhibit murmurs. (Sisson D,1987) The mitral valve is most commonly affected. Proliferation of the loose, fibroblastic tissue in the spongiosa of the valve in addition to a deposition of glycosaminoglycan occurs. There is also degeneration of the fibrosa layer in the valve. These chronic changes cause nodules to form on the valve leaflets which may progress and alter the function of the mitral valve structures. (O'Grady MR, 1995) The presence of these nodules prevents normal apposition of the valve leaflets and predisposes to mitral regurgitation. Because the valve is now insufficient, a portion of the blood from the left ventricle will flow in the direction of least resistance into the left atrium. The left atrium is compliant, and the chronic volume overload results in dilation. Compensatory mechanisms secondary to the mitral regurgitation include increases in both preload and systemic vasoconstriction. Increases in systemic vascular resistance worsen the pressure gradient between the left ventricle and the aortic outflow, enhancing the flow of blood into the

left atrium. This cycle causes greater dilation of the mitral valve and progression of the disease. A chronic LV volume overload results from elevation of left atrial pressures, predisposing to eccentric hypertrophy. Finally, the increase in pressure in the left atrium is reflected in the pulmonary tree, and pulmonary congestion occurs. Acute changes in blood pressure may cause a rapid onset of clinical signs without evidence of the chronic changes just described. Clinical signs are generally not appreciated until most dogs are 10 years of age or older. (O'Grady MR, 1995) Coughing is common and often occurs as a result of pressure on the left mainstem bronchus caused by left atrial enlargement. Other signs include respiratory dyspnea, exercise intolerance, syncope, and abdominal distension. In early stages of the disease, a soft, systolic murmur may be heard at the cardiac apex on the left side of the thorax. Over time, this may progress to a holosystolic murmur that radiates to the right side. Differentiating tricuspid insufficiency from a radiating mitral murmur may be difficult. The presence of jugular pulses may help distinguish tricuspid disease. (O'Grady MR, 1995) Irregular cardiac rhythms, pulse deficits, and poor pulses may be noted, but, generally, a sinus arrhythmia is present. (Bonagura, 1994) Lung sounds will vary from normal to diffuse crackles depending on the stage of the disease. Increased bronchial vesicular sounds are common in the older patient and may be difficult to differentiate from the early pathology of heart failure.

The electrocardiogram from affected dogs may show wide P waves as well as wide and tall QRS complexes. Arrhythmias, including supraventricular tachycardia or atrial premature depolarizations, can be seen, although most dogs have a sinus arrhythmia. Thoracic radiographs are essential to rule out collapsing airway disease, which is also very common in geriatric, small- breed dogs. Thoracic radiographs are also used to help rule out diseases and to determine the degree of pulmonary venous distension and pulmonary edema. Radiographic features of chronic mitral regurgitation include venous engorgement, left atrial enlargement, mainstem bronchial displacement, LV enlargement, loss of caudal cardiac waist, and pulmonary edema. Early pulmonary edema may appear as an interstitial pattern similar to an infectious etiology; the response to diuretics may help in differentiation. Progression of disease results in the typical alveolar edema with the characteristic perihilar distribution. Echocardiography is helpful in evaluating patients with chronic mitral regurgitation but is usually not necessary. Echocardiographic changes consistent with mitral regurgitation include left atrial dilation, thickened hyperechoic valve leaflets, exaggerated motion of the LV wall, and increased fractional shortening. Echocardiographic examination may be helpful in distinguishing degenerative valvular disease from infectious and neoplastic lesions and in confirming chordal rupture. Doppler evaluation is helpful in identifying subtle valvular lesions and in approximating severity. Other diseases to be ruled out by echocardiography that cause mitral regurgitation

include both dilated and hypertrophic cardiomyopathy. Chronic management of chronic mitral regurgitation includes preload reduction and arteriolar dilation. Antiarrhythmics may be needed to control supraventricular arrhythmias. The value of positive inotropic agents is unclear (Bright and Mears, 1997).

1.2.5 Epidemiology of cardiac diseases

Heart disease is a common cause for morbidity and mortality in dogs. Recent studies indicate that heart disease accounts for approximately 8% of overall mortality in dogs ,10 years of age. Heart disease is the fourth greatest contributor, after neoplastic disease, trauma, and ‘‘no diagnosis.’’ (Bonnett et all, 2005)

Pederson et al. (1999) reported in a study that at three years of age, approximately 90% of CKC spaniel has echocardiographic evidence of Mitral valve prolapse. Haggstrom (1996) and Pedersen et al. (1999) reported that the prevalance of regurgitant murmurs in CKC spaniel increases from less than 10% in oneyear- old dogs to 50% in six-year-old dogs and to more than 90% in dogs older than ten years.

Sleeper et al. (2002) reported that DCM an adult onset disease, with the exception of the Portuguese Water Dog (develops within the first weeks or months of life).

Broschk and Distl (2005) observed that the age of onset of DCM varies between 3 and 7 years of age. A juvenile form of DCM has been found in Portuguese Water Dogs and Doberman Pinscher Dogs.

Munday et al. (2006) reported the average age of 4 years for DCM. Sykes (2006) in a study of 71 dogs with possible or definite infective endocarditis observed that most affected dogs were of large breeds, and > 75% were older than five years. The aortic valve was affected in 36 of the 71 (51%) dogs, and the mitral valve was affected in 59%. Wess et al. (2010) reported DCM prevalence in various age groups was as follows: age group 1 (1 to <2 years) 3.3%, age group 2 (2 to <4 years) 9.9%, age group 3 (4 to <6 years) 12.5%, age group 4 (6 to <8 years) 43.6%, and age group 5 (>8 years) 44.1%. The cumulative prevalence of Doberman Pinscher cardiomyopathy was 58.2%.

1.2.6 Clinical symptoms in cardiac diseases

Wright et al. (1996) reported that animals presenting with cardiac diseases may have a history of syncope/lethargy (due to reduced cerebral perfusion), Weakness / reduced stamina (due to reduced skeletal muscle perfusion), dyspnea /wheeze /nocturnal cough/ orthopnea (due to pulmonary edema or pleural effusion associated with raised filling pressures resulting in congestive heart failure), abdominal distension (due to ascites, hepatomegaly or splenomegaly), pallor/ cold extremities and prolonged capillary refill time (due

to inadequate blood supply to the tissues and the body compensates by peripheral vasoconstriction so that blood can be supplied to vital organs of the body), exercise intolerance (due to inadequate blood supply to the tissues) and cardiac cachexia (severe wasting that occurs in association with chronic congestive heart failure as a result of poor tissue perfusion, cellular hypoxia, malabsorption, and anorexia). Gelzer (2002) observed that the atrial fibrillation is a commonly diagnosed supraventricular arrhythmia in older dogs and cats. Atrial fibrillation is usually associated with underlying heart disease - advanced stages of atrial enlargement secondary to dilated or hypertrophic cardiomyopathy or volume overload from chronic atrioventricular valve regurgitation. Atrial fibrillation may also occur in the absence of organic heart disease and is referred to as idiopathic or lone atrial fibrillation.

Dukes-McEwan et al. (2003) concluded that clinical signs in dogs with DCM and CHF include breathlessness or dyspnea, cough, depression, exercise intolerance, inappetence, syncope, weight loss, abdominal distention, and polydipsia. Clinical examination commonly reveals dyspnea, tachypnea, rales, crackles and increased breath sounds, tachycardia, arrhythymia, and, in some dogs, a systolic murmur of low to moderate intensity (grade I-III/VI).

Bright et al. (2005) reported that atrial fibrillation (AF) is the most common chronic pathologic arrhythmia in dogs. Although this arrhythmia typically occurs secondary to serious underlying cardiac disease, AF may occur spontaneously without an identifiable underlying cause in large and giant breed

dogs (lone AF). In dogs with underlying heart disease, the onset of AF usually coincides with a deterioration in clinical status (such as onset of weakness, anorexia, syncope, or congestive heart failure), and AF is associated with a high mortality rate in these patients. The long-term effects of lone AF in dogs are not clear at this time. However, it is possible that in affected dogs, wide fluctuations of heart rate with peak and mean heart rates exceeding those that occur during sinus rhythm may produce and advance cardiomyopathy.

Bomassi (2007) reported that the dogs having mitral valve disease showed increased capillary refill time, effort intolerance, dyspnoea with elevated respiratory rates, thread femoral pulse and pulse deficit, increased heart rates (about 180-190 beats /minute), irregular rhythm, a high intensity holosystolic left sided apical heart murmur.

1.2.7 Therapeutic management of cardiac diseases

Tidholm (2006) studied DCM initially treated with digoxin (mean dose 0.009 mg/kg per day) and furosemide (mean dose 3.6 mg/kg per day) with propranolol (mean dose 2.4 mg/kg per day) added after signs of CHF in 62 dogs was resolved, approximately one week after initial presentation, this dosage regimen was well tolerated and median survival time in dogs were 126 days, with a survival rate of one yr in 34%.

Carnieto Jr et al. (2009) have found beneficial effects of rofecoxibin limiting myocardial necrosis in dog in an experimental study. Beta-blockers have a long history in the treatment of hypertension and cardiac dysfunction, with more than 40 years of clinical use (Frishman, 2007). Carvedilol is a third-generation, vasodilatory betablocker that nonselectively blocks both the beta-1 and beta-2 adrenergic receptors and, in addition, has alpha-1 adrenergic receptor-blocking activity. Unlike traditional beta-blockers (eg, atenolol, metoprolol, and propranolol) that lower blood pressure by reducing cardiac output (Packer, 1998), vasodilatory beta-blockers can lower blood pressure by reducing systemic vascular resistance (SVR) (Sundberg et al.,1987). As with other beta-blockers, carvedilol has been shown to reduce sympathetic nervous system (SNS)-mediated cardiac stress and myocardial hypertrophy (Toda 2003). These activities likely contribute to the clinical benefits observed in patients treated with carvedilol for hypertension, heart failure, and post-MI left ventricular dysfunction (LVD). Carvedilol has also demonstrated antioxidant effects possibly attributable to stimulation of endothelial nitric oxide production or reduced nitric oxide inactivation (Toda, 2003). Furthermore, carvedilol may protect against reactive oxygen species (ROS) through scavenging of free radicals, suppression of free radical generation, and prevention of ferric ion-induced oxidation (Toda 2003; Dandona et al., 2007). Regular physical activity is an established protective factor for the prevention and treatment of leading non-communicable diseases, including cardiovascular disease and diabetes.

The benefits of dog ownership on physical activity levels are widely reported (Westgarth et al., 2014).

1.2.8 Nutraceutical management of cardiac diseases

A variety of nutritional deficiencies, dietary protein, fat, vitamins, minerals and trace elements are known to cause cardiac disease in various species. Nutritional management of dogs with cardiac problems can be handle with providing adequate calories, protein and modulating cytokine production to manage the possible cause of decreased appetite of the side effects of medications. A number of diseases affecting the heart are prevalent in canines. Acquired diseases, those which develop over the course of an animal's lifetime (rather than congenital defects present at birth), have recently been the subject of several studies to determine the efficacy of dietary supplementation on symptom presentation, disease severity, and mortality rates. Specifically, coenzyme Q10 (CoQ10), vitamin E (as alpha-tocopherol), L-carnitine, taurine, fish oil (omega-3 fatty acids), and arginine have all been evaluated in the prevention and treatment of many types of heart disease in dogs (Dove, 2001). Appropriate levels of certain dietary nutrients have been shown to increase life span, improve life quality, reduce symptoms and physical evidence of disease, and decrease mortality rates in these animals. (Devi and Jani, 2009)

Coenzyme Q 10

Mortensen (1993) have demonstrated CoQ 10's ability to protect heart tissue from functional and structural changes resulting from ischemia and reperfusion in both in vitro and in vivo studies. The rate and magnitude of clinical improvement appears to be directly related to the use of higher doses of CoQ 10 (Langsjoen and Langsjoen, 1999).

Vitamin E

Sebbag et al. (1994) reported that alpha-tocopherol prevent lethal ventricular arrhythmias associated with ischemia and reperfusion. The addition of vitamin E to the diet of dogs @ 40 IU/kg/day over a period of four months prevented volume overload-induced decrease in myocardial contractility and increased cardiac antioxidant reserves and glutathione peroxidase activity (Prasad et al.,1997).

L-carnitine

Keene et al. (1991) reported improved myocardial health on inclusion of L-carnitine in diet of Boxer dogs suffering from DCM.

Taurine

Orlova et al. (1991) reported that taurine supplementation, at 100 mg/ kg/ daily over the course of a month, reduced mortality rates, improved clinical condition, and enhanced myocardial contractility in dogs with congestive heart failure as compared to controls.

Omega-3 fatty acids

Freeman et al. (1998) have studied to test the ability of omega-3 fatty acids to reduce cytokines and improve clinical outcome in dogs with naturally occurring heart disease. Fish oil supplementation markedly improved cachexia and decreased interleukin-1 beta concentrations in dogs treated with fish oil as compared with the placebo group.

Arginine

Arginine (Arg), or L-arginine as it is called for its L-structure, is classified as semiessential or conditionally essential amino acid depending on health status or the lifecycle of the individual. Healthy adults can synthetize arginine and supply their own requirement, whereas it has to be supplemented in the growing organisms, after trauma or during various disease states (Tapiero et al., 2002;

Heird, 1998). L-arginine regulates multiple metabolic pathways involved in the metabolism of fatty acids, glucose, amino acids and proteins through the enhanced synthesis of cell-signaling molecules (e.g., nitric oxide, carbon monoxide, polyamines, cGMP and cAMP), as well as the increased expression of genes that promote whole-body oxidation of energy substrates (e.g., glucose and fatty acids). The main importance of arginine is attribute to its role in metabolism and physiology as a precursor for the synthesis of nitric oxide (NO), creatine, polyamines, and other molecules involved in the functions of multiple systems (Morris, 2007; Wu, 2009). Among these metabolites, NO is an almost ubiquitarian mediator formed through the action of the enzymes family known as NO synthase (NOS). NO acts as a neurotransmitter and a mediator of host defense, it regulates endothelium-dependent relaxation of blood vessels and energy metabolism and is considered a key signaling molecule involved in many biological processes (Dai et al., 2013; Gornik et al., 2004; Wu & Meininger, 2009). Polyamines are required for the synthesis of DNA and proteins, as well as for the cell proliferation and differentiation (Agostinelli, 2014). Proline is a major amino acid for collagen synthesis playing a key role in remodeling the extracellular matrix (Phang et al., 2012). Creatine is an antioxidant and participates in energy metabolism in skeletal muscle and nerves (Brosnan et al., 2007).

Aim of the study

L-arginine is an essential precursor for the production of nitric oxide (NO) which mediates the protective effects of the intact endothelium, acting as a vasodilator and endogenous antiatherogenic factor (Bohme et al., 1993; Paakkari and Lindsberg, 1995). Furthermore, NO plays an important role in the regulation of the renal blood flow, glomerular filtration rate and saline homeostasis (Bonomini et al., 2008). The effects of arginine administration have been investigated in humans affected by cardiovascular diseases (Pezza et al., 1998). In hypertensive patients under pharmacological therapies, oral administration of arginine for six weeks resulted in a reduction in systolic and diastolic blood pressure (Pezza et al., 1998).

At the present time, there is a paucity of data about the role of L- arginine in chronic cardiovascular and kidney disease in dogs. On the basis of the current literature, the objective of this research was to assess the effectiveness of oral L-arginine (integrated feed) in dogs with chronic cardiovascular and kidney diseases by assessing some clinical and ematochemical parameters that are usually altered in animals affected by these chronic diseases.

2. MATERIALS AND METHODS

2.1 Animals

A total of 120 dogs, 60 animals affected by chronic kidney disease and 60 subjects suffering from chronic cardiac disease, were included in the study with the informed owner consent. All the dogs were selected among the patients referred at the Veterinary Teaching Hospital of the University of Camerino.

2.1.1. Dogs with chronic kidney disease

The study included 60 dogs with kidney diseases (group K), 32 males and 28 females, mean age 9.9 years, mean weight 16.1 kg. The dogs were defined as having CKD (Group K) based on the results of medical history, clinical examination, blood pressure measurement, imaging, blood chemistry, and urinalysis. The main clinical findings included clinical signs compatible with CKD and typically a history of polyuria / polydipsia, weight loss, dysorexia, chronic vomiting, lethargy / asthenia, pallor of mucous membranes, edema and arterial hypertension. Diagnostic imaging and in particular renal ultrasonography revealed signs of chronic kidney disease such as diminished size, increased echogenicity with reduced cortical / medullary distinction, changes in shape and surface, presence of mineralized cystic lesions and / or

disseminated lesions and reduction of Doppler parenchymography. The diagnosis of CKD was then confirmed by biochemical findings such as serum creatinine concentration> 1.40 mg / dl with inadequate urinary specific gravity (<1030). All the animals included in the study showed clinical signs and ematochemical evidence of CKD for at least 3 months from the beginning of the study.

2.1.2 Dogs with cardiovascular diseases

The study included 60 dogs with cardiac diseases (Group C), 25 males and 35 females, mean age of 9.7 years, mean weight 20.1 kg. Dogs were included in the study after being examined on the basis of physical examination, clinical examination, blood chemistry, blood pressure (B.P), electrocardiography (ECG). Dogs exhibiting the symptoms of exercise intolerance, cold extremities, dyspnoea, tachypnoea (>30 acts/min), tachycardia (>11 bpm) jugular distension and pulsation, and coughing were included in the study.

2.2 Experimental design

The experimental design of the study included 60 dogs with kidney diseases (group K) receiving a conventional therapy consisting of Fortekor 0.25mg /kg and ranitidine 6.6 mg/Kg and 60 dogs with cardiac disease (group C) receiving

a conventional therapy consisting of diuretics, vasodilators and ace inhibitors. Group K was divided into two subgroups with 40 dogs treated with l-arginine (group K_a) and 20 dogs used as controls (group K_b). Group K_a consisted of 21 males and 19 females with a mean age of 9.7 years and mean weight of 21.7 kg. Group K_b included 11 males and 9 females with a mean age of 9.9 years and mean weight of 16.1 kg (Table 3).

Group C was divided into two subgroups, as well, with 40 dogs treated with l-arginine (group C_a) and 20 dogs used as controls (group C_b).

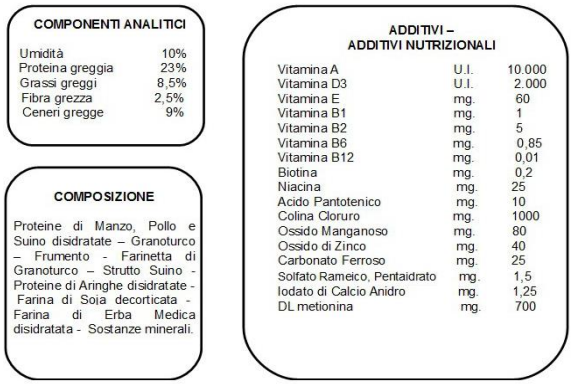
Group Ca was composed of 19 males and 21 females with a mean age of 9.7 years and mean weight of 20.9 kg. The group C_b was composed of 6 males and 14 females with a mean age of 9.5 years and mean weight of 18.8 kg (Table 3).

		Group K		Group C	
		K_A	K_B	C_A	C_B
SEX	M	21	11	19	6
	F	19	9	21	14
AGE		9.7±1.5	9.9±1.3	9.7±2	9.5±1.6
WEIGHT		21.7±4	16.1±4.2	20.9±4	18.8±5.2

Table 3 Characteristics of dogs with kidney disease (Group K) divided into experimental (Group Ka) and control (Group Kb) groups. Characteristics of dogs with cardiac disease (Group C) divided into experimental (Group Ca) and control (Group Cb) groups.

All dogs included in the study were fed twice a day with Nutrix più® maintenance feed 10% of their body weight. The Nutrix feed had the following

formulation: crude protein 23%, humidity 10%, crude fat 8.5%, crude fiber 2.5%, crude ash 9% (Figure 6).



COMPONENTI ANALITICI

Umidità	10%
Proteina greggia	23%
Grassi greggi	8,5%
Fibra grezza	2,5%
Ceneri gregge	9%

COMPOSIZIONE

Proteine di Manzo, Pollo e Suino disidratate – Granoturco – Frumento - Farinetta di Granoturco – Strutto Suino - Proteine di Aringhe disidratate - Farina di Soja decorticata - Farina di Erba Medica disidratata - Sostanze minerali.

ADDITIVI – ADDITIVI NUTRIZIONALI

Vitamina A	U.I.	10.000
Vitamina D3	U.I.	2.000
Vitamina E	mg.	60
Vitamina B1	mg.	1
Vitamina B2	mg.	5
Vitamina B6	mg.	0,85
Vitamina B12	mg.	0,01
Biotina	mg.	0,2
Niacina	mg.	25
Acido Pantotenico	mg.	10
Colina Cloruro	mg.	1000
Ossido Manganoso	mg.	80
Ossido di Zinco	mg.	40
Carbonato Ferroso	mg.	25
Solfato Rameico, Pentaidrato	mg.	1,5
Iodato di Calcio Anidro	mg.	1,25
DL metionina	mg.	700

Figure 6 Nutrix più mantenimento® food composition and nutritional additives

Galenic L-arginine tablets were provided by the Monzali pharmacy (Fabriano, Italy). The concentration of L-Arginine was 1gr / cpr. The tablets were administered to dogs belonging to the experimental groups (Group K_a and Group C_a) together with food at a dosage of 1gr / 20kg of weight.

L-arginine was administrated to the Group K_a and Group C_a for 180 days. Clinical examination, blood sampling and ultrasound monitoring were performed before starting the treatment (T0), after 15 days (T15), after 30 days (T30), after 90 days (T90), after 180 days (T180) and after 270 days (T270). All the dogs included in the study, both experimental and control groups, continued the conventional therapy for cardiac and kidney diseases throughout the experimental period.

For each animal, individual clinical boards were used in order to report the observed clinical data.

The owners were asked to fill out a questionnaire every day to evaluate the dog's behavior (Figure 7).

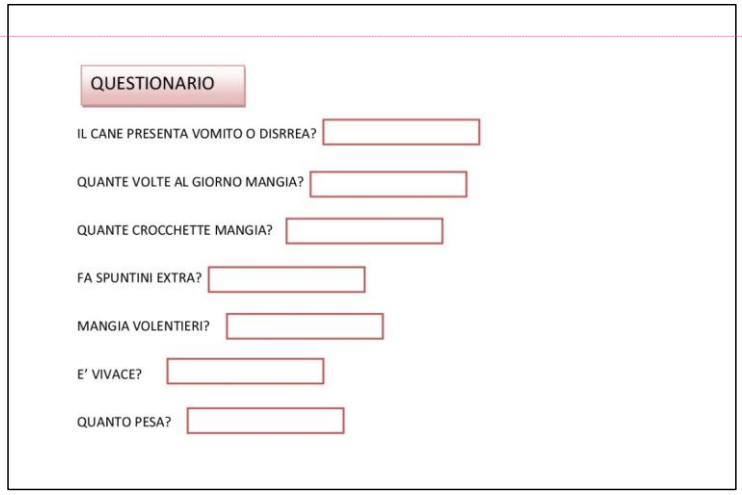


Figure 7 Questionnaire for owners

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All treatments, housing and animal care were carried out in accordance with the standards recommended by the EU Directive 2010/63/EU for animal experiments.

2.3 Hematological analysis

Blood samples were taken from the cephalic vein, 1 ml venous blood was collected into tubes containing EDTA (VENOSAFE Terumo® vacuum

system) and 5 ml into tubes containing clot-activators (VENOSAFE Terumo® vacuum system). After collection all samples were delivered to the laboratory and analyzed within an hour.

Blood samples collected in tubes with EDTA were processed for CBC. Erythrocytes number (RBC / mm3), hematocrit value (Hct%), total hemoglobin (Hgb g%), average corpuscular volume (MCV fl), average hemoglobin content (MCH pg), average corpuscular concentration of hemoglobin (MCHC g%), amplitude of erythrocyte distribution (RDW), total leukocytes (WBC / mm3), platelets (PLT / mm3), mean platelet volume (MPV), absolute reticulocyte count and percentage, index of reticulocyte production and leukocyte formula were assessed by using an automated analyzer (IDEXX procyte DX, IDEXX Laboratories Italia, S.r.l. , Milano, Italy). Blood samples collected in tubes containing clot activators were centrifuged for 10 minutes at 3000 × g to obtain sera (Hettich Rotina 46R, Tuttlingen, Germany). Sera obtained from Group K were tested for creatinine, urea and total proteins (BT 3500 VET, Biotecnica Instruments, Rome, Italy). Sera obtained from Group C were tested for CK, phosphorus, calcium, sodium, potassium, chlorine (BT 3500 VET, Biotecnica Instruments, Rome, Italy).

2.4 Diagnostic imaging in dogs with kidney disease

The ultrasound assessments of kidneys in dogs from Group K were obtained at the Veterinary Teaching Hospital of the University of Camerino by using the Esaote myLab ultrasound system. Ultrasound aspects of both kidneys were assessed at each time point in dogs from Group K.

The trichotomy of the abdomen up to the height of the coastal processes of the vertebrae was performed to facilitate the transmission of ultrasound, and gel was applied to guarantee better contact between the probe and the skin. A 7.5 MHz microconvex probe was used with the patients positioned in lateral decubitus on a perforated table by placing the transducer under the table through the hole (Nyland et al., 1995).

2.5 Electrocardiography and blood pressure assessment in dogs with cardiac disease

Cardiac function was assessed at each time point in dogs from Group C by standard ECG recorder using a Cardiart-6108-BPL (Service power supply Class 1, 220-240 V ± 10%; 50-60 Hz). The recording ECG was performed without the use of sedative drugs. ECG was recorded at paper speed of 50 mm /seconds and 25 mm/seconds with sensitivity of (1 cm=1 mV) without use of filter.

The ECG was recorded in standard body position (Tilley, 1985) with animals restrained in right lateral recumbency on the electrically insulated table positioned parallel to each other and perpendicular to the long axis of body and keeping head and neck flat on the table.

The electrodes were attached to the animal's skin after application of rubbing alcohol. The right forelimb (RA)(red electrode) and left forelimb (LA)(yellow electrodes) electrodes were placed proximal to the olecranon on the caudal aspect of the respective forelimbs; and the right hind limb (RF)(black electrode) and left hind limb (LF)(green electrodes) electrodes were placed over the patellar ligament on the anterior aspect of the respective hind limbs (Tilley, 1985).

A minimum of 10 complex in bipolar limb leads (I, II, III) and augmented unipolar limb leads (aVR, aVL and aVF) were recorded after comfortable setting of the dogs on the table on thermosensitive BPL 20 meter paper roll 1209X150 (A5).

Blood pressure was recorded by Non-invasive blood pressure (NIBP) instrument using pediatric cuff from radial artery. The first measurement was discarded and the average of 5-7 consecutive consistent indirect measurements were obtained (William DL et All, 2010).

2.6 Statistical analysis

Data were expressed as mean ± standard deviation of the mean (SEM). Two-way repeated measure ANOVA was applied to assess significant effects of L-arginine oral administration on studied parameters. When significant differences were found Tuckey's post hoc comparison was applied. P values <0.05 were considered statistically significant. Statistical analysis was performed using the Graphpad PRISM 9 software package.

3. Results

The animals included in the study maintained a good appetite and showed no significant deterioration during the experimental period. The owners reported a good appetite and an improvement in physical exercise of their dogs.

Statistical analysis revealed significant changes on UREA and blood pressure values experimental group (Ka and Ca) during the experimental period.

A significant decrease (P=0.0012) was found in UREA concentrations of dogs from Group K_a at T180 vs T0, T15, T30 and T90, and T270 vs T0 (Figure 8).

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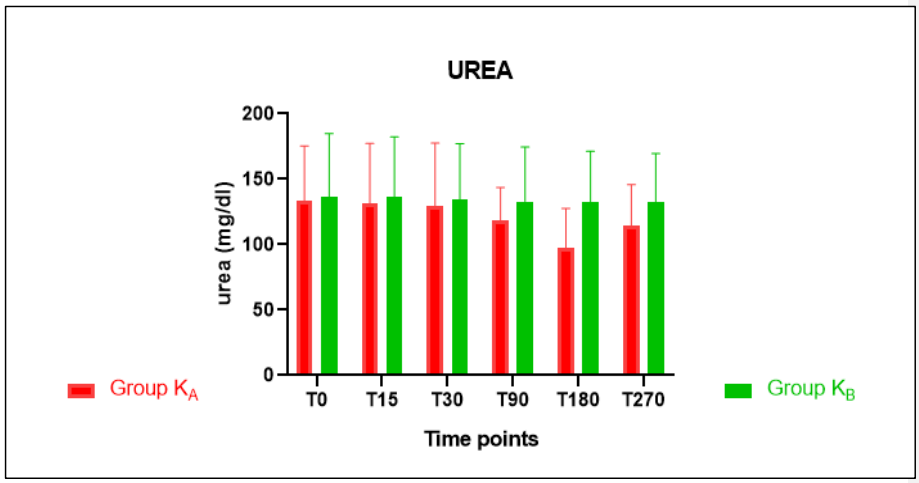

Figure 8 Concentrations of urea (mg/dl) found in treated dogs (K_a Group) and in control dogs (K_b group) during the experimental period (time points).

A significant decrease (P <0.0001) was found in systolic pressure of dogs from Group C_a during the experimental period. Significant differences were found at T0 VS T30, T90, T180 and T15 VS T30, T90, T180 and T30 VS T90 and T180 and T180 VS T270 (Figure 9).

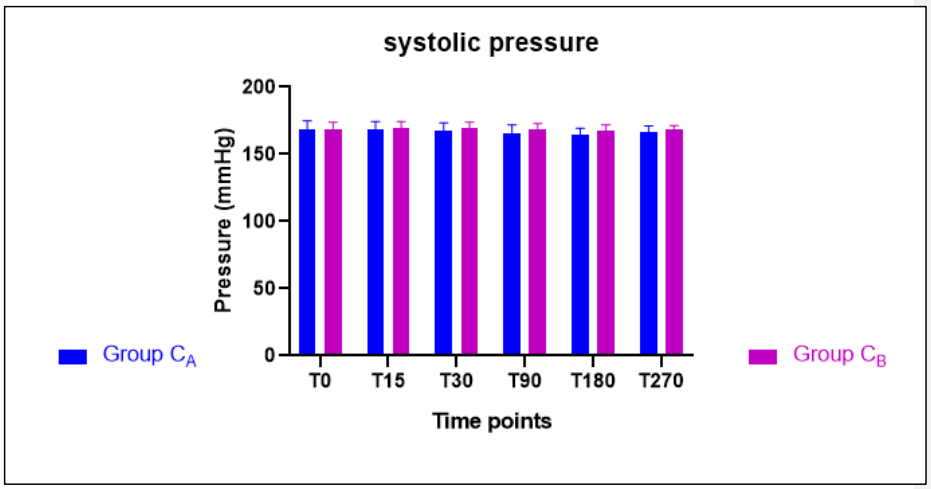

Figure 9 Systolic pressure (mmHg) observed in treated dogs (C_a Group) and in control dogs (C_b group) during the experimental period (time points).

A significant decrease (P <0.0001) was found in diastolic pressure within the Group C_a at T0 VS T30, T90, T180, at T15 VS T90, T180, at T30 VS T90, T180 and T180 VS T270 (Figure 10).

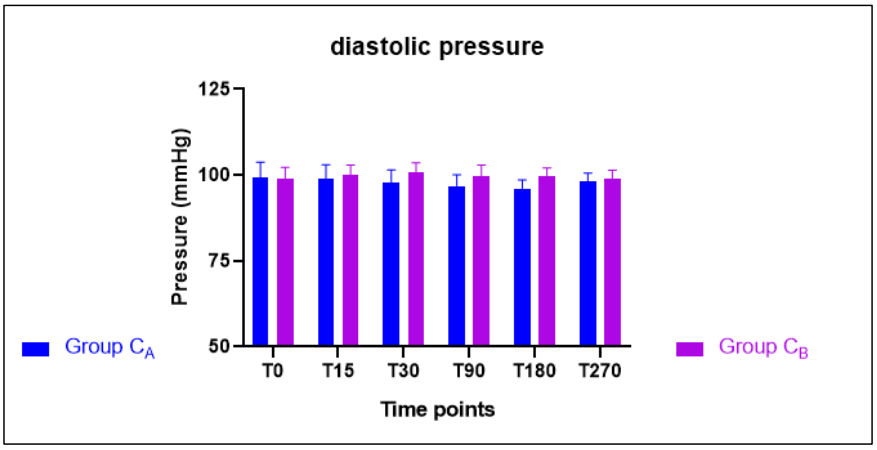

Figure 10 Diastolic pressure (mmHg) observed in treated dogs (C_a Group) and in control dogs (C_b group) during experimental period (time points).

4. Discussion

The aim of our study was to investigate the effects of dietary supplementation with L-arginine in dogs affected by CKD and cardiomyopathies.

Although at present there are no treatments for renal lesions related to CKD, it is often possible to resort to supportive and symptomatic therapies to improve the clinical and biochemical consequences of reduced kidney function, improving quality of life for affected animals.

Apart from conventional drug therapies, in recent years a careful attention to diet was applied to treat dogs and cats suffering from chronic kidney and cardiac diseases. Although sodium restriction is the most frequently suggested nutritional modification in dogs with heart disease (and sometimes the only one), it can be useful in these animals to correct a great variety of nutrients. Research is now beginning to show that dietary factors can be able to modulate these pathologies, both slowing down their progression and minimizing the number of drugs required, and certainly improving dogs' quality of life.

High blood pressure can be a cause or consequence of CDK. Its presence can negatively affect the long-term prognosis. This is a common complication of CDK, reported in 66% of affected cats. Although a recent study suggests that hypertension in the CKD dog is unusual, others have documented an incidence of 30 to 93%, therefore dogs suffering from glomerular pathology have a higher risk of hypertension (Ettinger, 2007).

In this study the administration of L-arginine to dogs affected by kidney disease determined a significant effect on renal function. In particular, a significant decrease in urea serum concentrations was found in experimental Group K_a compared to control Group K_b . In our study the administration of L-arginine was effective in improving biochemical parameters such as UREA in subjects with CKD. After 30 days of L-arginine administration, dogs from Group K_a were more reactive with better appetite with the mucous membranes and heart rate were improved at the clinical visit.

Other studies demonstrated the importance of NO and L-arginine oral administration in modulating the activity of the peripheral renin-angiotensin system during the evolution of renovascular hypertension (Hidemoto, 1994).

Most authors believe that the CKD is characterized by a reduced production of NO. This may result from the presence in the circulation of inhibitors of NOS enzyme such as asymmetric dimethylarginine (ADMA). Accumulation of this endogenous eNOS inhibitor may result in reduced effective plasma renal flow and increased renal vascular resistance and blood pressure (Kielstein JT et al., 2003). ADMA is a product of protein turnover that is eliminated by degradation by the enzyme dimethylarginine dimethylaminohydroxylase (DDAH) and, to a lesser extent, by renal excretion. In uremic patients, the plasma concentration of ADMA is increased (Vallance et all., 1992). The accumulation of ADMA in IRC could also be accentuated by acidosis, since the optimal pH for DDAH enzyme activity is 7.4 (Knipp et all., 2003), and, moreover, we have seen that

the pH intracellular also modulates the activity of the nNOS and iNOS enzymes (Gorren et all, 1998). From the studies reported up to now on the potential role of NO in clinical nephrology, two possibilities emerge: 1) nephrotoxic role (insufficient bioavailability of NO); 2) nephroprotective role (adequate bioavailability of NO). In the first case the progression of renal damage towards CKD is accelerated and is accompanied by extrarenal vascular damage with arterial hypertension. In the second case the progression of renal damage is slowed down and is not associated with arterial hypertension. These studies are still predominantly experimental and clinical observations in the medium-long term are necessary for a better definition of NO / renal function relationships. The study of the double action of NO, depending on the degree of availability, is stimulating and of considerable clinical interest. The possibility of having simple and at the same time reliable diagnostic means for assessing the bioavailability of NO will help to define the role of this radical in chronic kidney disease. At the same time, it seems desirable to develop therapeutic measures capable of effectively antagonizing the NO deficiency, currently not yet available for current clinical use. Devi and Jani (2009) based on the findings of endothelial dysfunction in patients with chronic heart failure (CHF), have begun to study the effects of arginine supplementation in a group of dogs. In normal patients, L-arginine supplementation was unlikely to have an effect on nitric oxide production because L-arginine improve endothelium-dependent vasodilation and cardiac output. L-arginine has been shown reduced heart rate

and systemic vascular resistance, with no negative effects on cardiac contractility or other echocardiographic variables. These studies show that the arginine supplementation may provide beneficial effects in patients with CHF. Several Authors demonstrated that renal dysfunction is characterized by a reduced production of NO. NO reduction may result from the presence of circulating inhibitors of the enzyme NOS such as ADMA (Bonomini et al., 2008; Kielstein et al., 2003). The accumulation of such endogenous inhibitor of NOS may result in reduction of renal plasma flow, increased vascular resistance on kidney and increased blood pressure (Kielstein et al., 2003). In a recent clinical trial, a short course of rosuvastatin treatment decreased ADMA levels in dogs affected by atrial fibrillation (Li et al., 2012). Due to the volatile and unstable nature of NO, its evaluation is better performed to indirectly measure the level of its metabolism product (Lau et al., 2000) study by Nakamoto in 1994 in dogs demonstrated the important contribution of L-arginine, administered orally, in modulating the increased activity of the renin-angiotensin system during evolution of renal vascular hypertension causing renal dysfunction. The effect of arginine supplementation was also evaluated in patients with myocardial infarction. The result was that in patients treated with arginine there was a lower incidence of deaths from cardiovascular death, re- infarction, pulmonary shock / edema and recurrent myocardial ischemia compared to patients who received placebo (Bednarz, 2005). In physiological conditions, the endothelium releases NO in response to vasodilation stimuli,

but in many cardiovascular diseases the function of the vascular endothelium is altered. It has therefore been hypothesized that the exogenous contribution of arginine could improve endothelial function in patients in whom this function was altered, but this turns out to be true only in those patients who have deficits of this amino acid (Arnal, 1995; Hishikawa K, 1992).

In our study the dogs belonging to the experimental group C_a showed a significant decrease in systolic and diastolic pressure after 30 days of L-arginine administration. Similar results were found in hypertensive human patients in a study by Pezza and colleagues (1998) who demonstrated that oral administration of arginine for six weeks resulted in a reduction in systolic and diastolic blood pressure. Furthermore, at the clinical visit dogs from group C_a showed lower respiratory rate and heart rate after 90days (T90) L-arginine treatment compared to T0 but still above the reference range.

Heart disease and nephropathy in dogs and cats are frequent and often incurable, our task is to try to improve the expectations and quality of pets' lives. Cardiovascular diseases are still one of the most common life-threatening disorders in dogs and cats. Most cardiac diseases in these animal species cannot be cured permanently and the pathological process is typically progressive, leading to advanced congestive heart failure or lethal cardiac arrhythmias.

Our results showed a general improvement in health conditions of dogs oral administration of L-arginine demonstrating how nutritional interventions for the treatment of heart and kidney diseases remain one of the cornerstones of

supportive therapy and one of the most stimulating possibilities for further scientific research.

5. References

- Adams, L.G. (2004). Chronic renal failure. In: The 5 minute veterinary consult.,4TH Edn. Edt. Tilly LP and Smith FWK, Lippincott William and Wilkins, Philadelphia, USA, pp: 1124-1125.
- Agostinelli E. (2014). Polyamines and transglutaminases: biological, clinical, and biotechnological perspectives. Amino Acids 46, 475–485. 10.1007/s00726-014-1688-0
- Aresu, L., Benali, S., Garbisa, S., Gallo, E. & Castagnaro, M. (2011). Matrix metalloproteinases and their role in the renal epithelial mesenchymal transition. Histol Histopathol, 26(3), pp. 307-13.
- Aresu, L., Benali, S., Garbisa, S., Gallo, E. & Castagnaro, M. (2011). Matrix metalloproteinases and their role in the renal epithelial mesenchymal transition. Histol Histopathol, 26(3), pp. 307-13.
- Atkins, C.E. (1991). The role of noncardiac disease in the devel- opment and precipitation of heart failure. Vet. Clin. North. Am. Small. Anim .Pract., 21: 1035–1080.
- Atkins, C.E. (2007). An approach to asymptomatic acquired heart disease in dogs. Proceedings of the World Small Animal Veterinary Association., Sydney (Australia).
- Barr, S.C. (1991). American trypanosomiasis in dogs. Compend. Contin. Ed., 13:745–752.

- Beckel, N. F., O'Toole, T. E., Rozanski, E. A., and Labato, M. A. (2005). Peritoneal dialysis in the management of acute renal failure in 5 dogs with leptospirosis. Journal of Veterinary Emergency and Critical Care, 15(3): 201-205.

- Beckel, N. F., O'Toole, T. E., Rozanski, E. A., and Labato, M. A. (2005). Peritoneal dialysis in the management of acute renal failure in 5 dogs with leptospirosis. Journal of Veterinary Emergency and Critical Care, 15(3): 201-205.

- Bednarz B, Jaxa-Chamiec T, Gebalska J, Herbaczynska-Cedro K, Ceremuzynski L. L-Arginine supplementation prolongs exercise capacity in congestive heart failure. Kardiol Pol. 2004;60:348–53.
- Bednarz B, Jaxa-Chamiec T, Maciejewski P, Szpajer M, Janik K, Gniot J, Kawka-Urbanek T, Drozdowska D, Gessek J, Laskowski H. Efficacy and safety of oral l-arginine in acute myocardial infarction. Results of the multicenter, randomized, double-blind, placebo- controlled ARAMI pilot trial. Kardiol Pol. 2005;62:421–7
- Benali, S.L., Lees, G.E., Castagnaro, M. & Aresu, L. (2014). Epithelial mesenchymal transition in the progression of renal disease in dogs. Histol Histopathol, 29(11), pp. 1409-14.

- Benali, S.L., Lees, G.E., Castagnaro, M. & Aresu, L. (2014). Epithelial mesenchymal transition in the progression of renal disease in dogs. Histol Histopathol, 29(11), pp. 1409-14.

- Benali, S.L., Lees, G.E., Nabity, M.B., Mantovani, R., Bonsembiante, F. & Aresu, L. (2013). De novo expression of human leukocyte antigen-DR (HLA-DR) and loss of beta-catenin expression in tubular epithelial cells: a possible event in epithelial-mesenchymal transition in canine renal diseases. Vet J, 198(1), pp. 229-34.
- Bertani, T., Cutillo, F., Zoja, C., Broggini, M. & Remuzzi, G. (1986). Tubulo-interstitial lesions mediate renal damage in adriamycin glomerulopathy. Kidney Int, 30(4), pp. 488-96.
- Bohme GA, Bon C, Lemaire M, Reibaud M, Piot O, Stutzmann JM, Doble A, Blanchard JC. Altered synaptic plasticity and memory formation in nitric oxide synthase inhibitor-treated rats. Proc Natl Acad Sci USA. 1993;90:9191–4.
- Bomassi, E. (2007).Case report: Mitral valve diseases in a large breed dog. Prat. Med. Chir. Anim.Comp., 42:123-130.
- Bonagura JD : Management of chronic canine heart disease, 65° CONGRESSO INTERNAZIONALE MULTISALA SCIVAC RIMINI 28-30 MAGGIO 2010
- Bonagura JD: Cardiovascular diseases. In Sherding RG (ed): The Cat: Diseases and Clinical Management, ed 2. New York, Churchill Livingstone, 1994, p 911

- Bonnett BN, Egenvall A, Olson P, Hedhammar A ˚ . Mortality in over 350,000 insured Swedish dogs from 1995–2000: I. Breed-,gender-, age- and cause-specific rates. Acta Vet Scand 2005;46: 105–120.
- Bonomini M, Sirolli V, Di Pietro N, Pandolfi A. Ridotta biodisponibilitá di ossido nitrico nell'insufficienza renale cronica: un nuovo fattore di progressione? Giornale Italiano di Nefrologia. 2008, 25 (3):306-16.
- Bovee, K.C., Joyce, T., Reynolds, R. & Segal, S. (1978). The fanconi syndrome in Basenji dogs: a new model for renal transport defects. Science, 201(4361), pp. 1129-31.
- Brambilla, P.G., Di marceloo,M. and Tradati, F. (2003). Complex congenital heart disease: prevalence and clinical findings. Vet. Res. Commun., 27(Suppl.1):735-738
- Brenner, B.M., Bohrer, M.P., Baylis, C. & Deen, W.M. (1977). Determinants of glomerular permselectivity: Insights derived from observations in vivo. Kidney Int, 12(4), pp. 229-37.
- Brenner, B.M., Meyer, T.W. & Hostetter, T.H. (1982). Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. N Engl J Med, 307(11), pp. 652-9.

- Bright J.M. and Mears E.: chronic heart disease and its management. veterinary clinics of north america,: small animal practice volume 27, number 6, november 1997
- Bright, J.M., Martin, J.M. and Mama, K. (2005). A retrospective evaluation of transthoracic biphasic electrical cardioversion for atrial fibrillation in dogs. J. Vet. Cardiol.,7: 85-96.
- Broschk, C. and Distl, O. (2005). Dilated cardiomyopathy (DCM) in dogspathological, clinical, diagnosis and genetic aspects. Dtsch. Tierarztl. Wochenschr., 112(10): 380-385.
- Brosnan ME, Brosnan JT. Orotic acid excretion and arginine metabolism. J Nutr. 2007;137(6 Suppl 2):1656S–1661S
- Brown N, Segev G, Francey T, Kass P, Cowgill LD. Glomerular filtration rate, urine production, and fractional clearance of electrolytes in acute kidney injury in dogs and their association with survival. Journal of Veterinary Internal Medicine, n. 29, p. 28–35, 2015
- Brown, S.A. (2008). Oxidative stress and chronic kidney disease. Vet Clin North Am Small Anim Pract, 38(1), pp. 157-66, vi.
- Brown, S.A., Finco, D.R., Crowell, W.A., Choat, D.C. & Navar, L.G. (1990). Single-nephron adaptations to partial renal ablation in the dog. Am J Physiol, 258(3 Pt 2), pp. F495-503.

- Brown, S.A., Finco, D.R., Crowell, W.A., Choat, D.C. & Navar, L.G. (1990). Single-nephron adaptations to partial renal ablation in the dog. Am J Physiol, 258(3 Pt 2), pp. F495-503
- Calvert CA: Update: Canine dilated cardiomyopathy. In Kirk RW, Bonagura JD (eds): Current Veterinary Therapy XI. Philadelphia, WB Saunders, 1992, p 773
- Cambridge, G., Mac Arthur, C.G.C., Waterson, A.P., Goodwin, J.F. and Oakley, C.M. (1979). Antibodies to Coxsackie B viruses in congestive cardiomyopathy. Br. Heart. J., 41:692–696.
- Carnieto, Jr, A Dourado, P.M.M., da Luz, P.L. and Chagas, A.C.P. (2009). Selective cyclooxygenase-2 inhibition protects against myocardial damage in experimentlischemia. Clinics., 64(3):245-52.
- Ceremuzynski L, Chamiec T, Herbaczynska-Cedro K. Effect of supplemental oral L-arginine on exercise capacity in patients with stable angina pectoris. Am J Cardiol. 1997;80:331–3.
- Chakrabarti, S., Syme, H.M. & Elliott, J. (2012). Clinicopathological variables predicting progression of azotemia in cats with chronic kidney disease. J Vet Intern Med, 26(2), pp. 27581.
- Chawla, L.S. & Kimmel, P.L. (2012). Acute kidney injury and chronic kidney disease: an integrated clinical syndrome. Kidney Int, 82(5), pp. 516-24.

- Chew, D.J., Dibartola S.P., Schenck P.A. (2011b). Acute Renal failure. In: Canine and Feline Nephrology and Urology, 2nd edition, Elsevier saunders publication, pp: 63-92.
- Chew, D.J., Dibartola, S.P., Schenck, P.A. (2011a).Chronic Renal failure. In: Canine and Feline Nephrology and Urology, 2nd edition, Elsevier saunders publication, pp: 145-196
- Cianciolo, R.E., Mohr, F.C., Aresu, L., Brown, C.A., James, C., Jansen, J.H., Spangler, W.L., van der Lugt, J.J., Kass, P.H., Brovida, C., Cowgill, L.D., Heiene, R., Polzin, D.J., Syme, H., Vaden, S.L., van Dongen, A.M. & Lees, G.E. (2016). World Small Animal Veterinary Association Renal Pathology Initiative: Classification of Glomerular Diseases in Dogs. Vet Pathol, 53(1), pp. 113-35
- Clark, W.F., Sontrop, J.M., Huang, S.H., Moist, L., Bouby, N. & Bankir, L. (2016). Hydration and Chronic Kidney Disease Progression: A Critical Review of the Evidence. Am J Nephrol, 43(4), pp. 281-92.
- Colville T., Bassert J. M.: Clinical Anatomy and Physiology for Veterinary Technicians Third Edition 2016 Elsevier
- Colville T., Bassert M.J.: Clinical Anatomy and Physiology for Veterinary Technicians Third Edition Elsevier 2016
- Cowgill, L. D., James, K.M., Levy, J.K., Browne, J.K., Miller, A., Lobingier, R.T., Egrie, J.C. (1998). Use of recombinant human erythropoietin for management of anemia in dogs and cats with renal

failure. Journal of American Veterinary Medical Association , 212(4): 521-528

- Cowgill, L.D., Polzin, D.J., Elliott, J., Nabity, M.B., Segev, G., Grauer, G.F., Brown, S., Langston, C. & van Dongen, A.M. (2016). Is Progressive Chronic Kidney Disease a Slow Acute Kidney Injury? Vet Clin North Am Small Anim Pract, 46(6), pp. 995-1013.
- Cozzolino, M., Funk, F., Rakov, V., Phan, O., and Teitelbaum, I. (2014). Preclinical pharmacokinetics, pharmacodynamics and safety of sucroferric oxyhydroxide. Current Drug Metabolism, 15(10): 953-965.
- Dai ZL, Li XL, Xi PB et al (2013) L-Glutamine regulates amino acid utilization by intestinal bacteria. Amino Acids 45:501–512
- Dambach, D.M., Lannon, A., Sleeper, M.M. and Buchanan, J. (1999). Familial dilated cardiomyopathy of young Portuguese water dogs. J. Vet .Intern .Med., 13:65–71.
- D'Amico, G. & Bazzi, C. (2003). Pathophysiology of proteinuria. Kidney Int, 63(3), pp. 809-25.
- Dandona, P., Ghanim, H., Brooks, D. P. (2007) . Antioxidant activity of carvedilol in cardiovascular disease. J. Hypertens., 25: 731–41.
- Davidson, A.G., Bell, R.J., Lees, G.E., Kashtan, C.E., Davidson, G.S. & Murphy, K.E. (2007). Genetic cause of autosomal recessive hereditary nephropathy in the English Cocker Spaniel. J Vet Intern Med, 21(3), pp. 394-401.

- Deen, W.M., Lazzara, M.J. & Myers, B.D. (2001). Structural determinants of glomerular permeability. Am J Physiol Renal Physiol, 281(4), pp. F579-96
- Deen, W.M., Maddox, D.A., Robertson, C.R. & Brenner, B.M. (1974). Dynamics of glomerular ultrafiltration in the rat. VII. Response to reduced renal mass. Am J Physiol, 227(3), pp. 55662
- Deen, W.M., Robertson, C.R. & Brenner, B.M. (1972). A model of glomerular ultrafiltration in the rat. Am J Physiol, 223(5), pp. 1178-83.
- Devi S and. Jani R.G: Review on Nutritional Management of Cardiac Disorders in Canines Veterinary World Vol.2, No.12:482-485, December 2009
- Dorval, P., Boysen, S. R. (2009). Management of acute renal failure in cats using peritoneal dialysis: a retrospective study of six cases. Journal of Feline Medicine and Surgery, 11: 107-115.
- Dove R.S. : Nutritional therapy in the treatment of heart disease in dogs. Altern Med Rev 2001; Sep 6 Suppl: S38-45
- Dove, R.S. (2001). Nutritional therapy in the treatment of heart disease in dogs. Alt. Med. Rev., 6: 38-45.
- Dukes-McEwan J, Borgarelli M, Tidholm A, Vollmar AC and Haggstrom J. Proposed guidelines for the diagnosis of canine idiopathic dilated cardiomyopathy. J Vet Cardiol 2003;5(2):7-19.

- Eddy, A.A. (2000). Molecular basis of renal fibrosis. Pediatr Nephrol, 15(3-4), pp. 290-301.
- Eddy, A.A. (2014). Overview of the cellular and molecular basis of kidney fibrosis. Kidney Int Suppl (2011), 4(1), pp. 2-8.
- Egenvall, A., Bonnett, B.N. and Hagstrom, J. (2006). Heart Disease as a Cause of Death in Insured Swedish Dogs Younger Than 10 Years of Age. J.Vet.Intern.Med., 20(4):894-903
- Elliott D. A. (2006). Nutritional Management of Chronic Renal Disease in Dogs and Cats. Veterinary Clinics Small Animal Practice, 36: 1377–1384.
- Finco, D.R. (2004). Association of systemic hypertension with renal injury in dogs with induced renal failure. J Vet Intern Med, 18(3), pp. 289-94.
- Finco, D.R., Brown, S.A., Brown, C.A., Crowell, W.A., Cooper, T.A. & Barsanti, J.A. (1999). Progression of chronic renal disease in the dog. J Vet Intern Med, 13(6), pp. 516-28.

- Finco, D.R., Brown, S.A., Brown, C.A., Crowell, W.A., Cooper, T.A. & Barsanti, J.A. (1999). Progression of chronic renal disease in the dog. J Vet Intern Med, 13(6), pp. 516-28.

- Finco, D.R., Brown, S.A., Cooper, T., Crowell, W.A., Hoenig, M. & Barsanti, J.A. (1994). Effects of parathyroid hormone depletion in dogs with induced renal failure. Am J Vet Res, 55(6), pp. 867-73.
- Freeman, L., Rush, J., Kehayias, J.J., Ross, J., Meydani, S.N., Brown, D.J., Dolnikowski , G.G., Marmor , B., White, C.M., Dinarello, C. and Roubenoff, R. (1998). Nutritional Alterations and the Effect of Fish Oil Supplementation in Dogs with Heart Failure. J. Vet. Intern. Med., 12: 440-448.
- Frishman, W.H. (2007). A historical perspective on the development of betaadrenergic blockers. J. Clin. Hypertens., 9(4 Suppl 3):19–27.
- Geigy, C. A., Schweighauser, A., Doherr, M. and Francey, T. (2011). Occurrence of systemic hypertension in dogs with acute kidney injury and treatment with amlodipine besylate. Journal of Small Animal Practice, 52(7): 340-346
- Gelzer, A (2002).The challenges of atrial fibrillation. Proc 20th Annual Forum ACVIM., 20: 92.
- Genovese, F., Manresa, A.A., Leeming, D.J., Karsdal, M.A. & Boor, P. (2014). The extracellular matrix in the kidney: a source of novel non-invasive biomarkers of kidney fibrosis? Fibrogenesis Tissue Repair, 7(1), p. 4.
- Gil, R.B., Ortiz, A., Sanchez-Nino, M.D., Markoska, K., Schepers, E., Vanholder, R., Glorieux, G., Schmitt-Kopplin, P. & Heinzmann, S.S.

(2018). Increased urinary osmolyte excretion indicates chronic kidney disease severity and progression rate. Nephrol Dial Transplant.

- Gornik H.L. ,Creager M.A. :Arginine and Endothelial and Vascular Health ;The Journal of Nutrition, Volume 134, Issue 10, October 2004, Pages 2880S–2887S
- Gorren AC, Schrammel A, Schmidt K, Mayer B. Effects of pH on the structure and function of neuronal nitric oxide synthase. Biochem J 1998; 331 (Pt 3): 801-7
- Grauer Gregory, F. (2009). Urinary Tract Disorders. In: Nelson, R. W. and C. G. Couto, Ed: Small Animal Internal medicine, 4th Ed. Elsevier, pp: 607-696.
- Häggström, J.(1996). Chronic valvular disease in Cavalier King Charles spaniels. Epidemiology, inheritance and pathophysiology. PhD Thesis. Swedish University of Agricultural Sciences, Uppsala, Sweden.
- Harold, D. (2005). Nursing management of the acute renal failure patient. Proceeding of the NAVC, North American Veterinary Conference, pp: 15-17
- Heird WC. Amino acids in pediatric and neonatal nutrition. Current Opinion in Clinical Nutrition and Metabolic Care. 1998; 1:73-78.
- Hoppe, A. & Karlstam, E. (2000). Renal dysplasia in boxers and Finnish harriers. J Small Anim Pract, 41(9), pp. 422-6.

- Jacob et al., 2002 Jacob F., Polzin D.J., Osborne C.A., Allen T.A., Kirk C.A., Neaton J.D., et al. Clinical evaluation of dietary modification for treatment of spontaneous chronic renal failure in dogs. Journal of the American Veterinary Medical Association 220(8): 1163-70, 2002
- Jacob, F., Polzin, D.J., Osborne, C.A., Neaton, J.D., Kirk, C.A., Allen, T.A. & Swanson, L.L. (2005). Evaluation of the association between initial proteinuria and morbidity rate or death in dogs with naturally occurring chronic renal failure. J Am Vet Med Assoc, 226(3), pp. 393-400
- Jacob, F., Polzin, D.J., Osborne, C.A., Neaton, J.D., Lekcharoensuk, C., Allen, T.A., Kirk, C.A. & Swanson, L.L. (2003). Association between initial systolic blood pressure and risk of developing a uremic crisis or of dying in dogs with chronic renal failure. J Am Vet Med Assoc, 222(3), pp. 322-9.
- Jobgen WS, Fried SK, Fu WJ, Meininger CJ, Wu G. Regulatory role for the arginine nitric oxide pathway in metabolism of energy substrates. Journal of Nutritional Biochemistry. 2006; 17:571–588
- Kamble, M.M and Vaidya, S.M. (2002). Effect of antihypertensive drugs on cardiac enzymes in hypertension with myocardial infarction in NIDDM. Ind. J. Clinic. Biochem., 17(2): 60-63

- Keene, B.W., Kittleson, M.D. and Rush, J.E. (1988). Frequency of myocardial carnitine deficiency associated with spontaneous dilated cardiomyopathy. Proc. Annu. Vet. Med. Forum., 6: 757.
- Keene, B.W., Panciera, D.P., Atkins, C.E., Regitz, V., Schmidt, M.J. and Shug, A.L. (1991). Myocardial L-carnitine deficiency in a family of dogs with dilated cardiomyopathy. J. Am. Vet. Med. Assoc., 198:647–650
- Keene, B.W., Panciera, D.P., Atkins, C.E., Regitz, V., Schmidt, M.J. and Shug, A.L. (1991). Myocardial L-carnitine deficiency in a family of dogs with dilated cardiomyopathy. J. Am. Vet. Med. Assoc., 198:647–650.
- Kielstein JT, Bode-Boger SM, Frolich JC, Ritz E, Haller H, Fliser D. Asymmetric dimethylarginine, blood pressure, and renal perfusion in elderly subjects. Circulation 2003; 107: 1891-5.
- Kogika, M.M., Lustoza, M.D., Hagiwara, M.K., Caragelasco, D.S., Martorelli, C.R. & Mori, C.S. (2015). Evaluation of oxidative stress in the anemia of dogs with chronic kidney disease. Vet Clin Pathol, 44(1), pp. 70-8.
- Kurtz, A. (2011). Renin release: sites, mechanisms, and control. Annu Rev Physiol, 73, pp. 37799.
- Knipp M, Braun O, Gehring PM, et al. Zn(II)-free dimethylargininase-1 (DDAH-1) is inhibited upon specific Cysnitrosylation. J Biol Chem 2003; 278: 3410-6.

- Kurtz, A., Eckardt, K.U., Neumann, R., Kaissling, B., Le Hir, M. & Bauer, C. (1989). Site of erythropoietin formation. Contrib Nephrol, 76, pp. 14-20; discussion 21-3.
- Langsjoen, P.H. and Langsjoen, A.M.(1999). Overview of the use of coenzyme Q10 in cardiovascular disease. Biofactors, 9:273-84.
- Langston C. Managing fluid and electrolyte disorders in renal failure. Veterinary Clinics of North America—Small Animal Practice, v. 38, n. 3, p. 677–697, 2008.
- Langston CE, Cowgill LD, Spano JA. A. Applications and outcome of hemodialysis in cats: a review of 29 cases. Journal of veterinary internal medicine / American College of Veterinary Internal Medicine, v. 11, n. 6, p. 348–355, 1997.
- Lash, J.P., Go, A.S., Appel, L.J., He, J., Ojo, A., Rahman, M., Townsend, R.R., Xie, D., Cifelli, D., Cohan, J., Fink, J.C., Fischer, M.J., Gadegbeku, C., Hamm, L.L., Kusek, J.W., Landis, J.R., Narva, A., Robinson, N., Teal, V. & Feldman, H.I. (2009). Chronic Renal Insufficiency Cohort (CRIC) Study: baseline characteristics and associations with kidney function. Clin J Am Soc Nephrol, 4(8), pp. 1302-11.
- Lau T, Owen W, Yu YM, et al. Arginine, citrulline, and nitric oxide metabolism in end-stage renal disease patients. J Clin Invest 2000; 105: 1217-25.

- Lazzara, M.J. & Deen, W.M. (2007). Model of albumin reabsorption in the proximal tubule. Am J Physiol Renal Physiol, 292(1), pp. F430-9
- Lee YJ, Chan JP, Hsu WL, Lin KW, Chang CC. Prognostic factors and a prognostic index for cats with acute kidney injury. Journal of Veterinary Internal Medicine, v. 26, n. 3, p. 500–5, 2012.
- Lefebvre, H.P., Brown, S. A., Chetboul, V., King, J.N., Pouchelon, J.L., Toutain, P.L., (2007). Angiotensin- converting enzyme inhibitors in veterinary medicine. Current Pharmaceutical Design, 13 (13): 1347-1361.
- Lehnhardt, A., Kemper, M. J. (2011). Pathogenesis, diagnosis and management of hyperkalemia. Pediatric Nephrology, 26(3): 377–384.
- Li J, Xia W, Feng W, Qu X. Effects of rosuvastatin on serum asymmetric dimethylarginine levels and atrial structural remodeling in atrial fibrillation dogs. Pacing Clin Electrophysiol. 2012, 35(4):456-64.
- Li, X., Pabla, N., Wei, Q., Dong, G., Messing, R.O., Wang, C.Y. & Dong, Z. (2010). PKC-delta promotes renal tubular cell apoptosis associated with proteinuria. J Am Soc Nephrol, 21(7), pp. 1115-24
- Limas, C.J. (1996).Autoimmunity in dilated cardiomyopathy and major histocompatibility complex. Int. J. Cardiol., 54:113–116.
- Lindstrom, K.E., Blom, A., Johnsson, E., Haraldsson, B. & Fries, E. (1997). High glomerular permeability of bikunin despite similarity in

charge and hydrodynamic size to serum albumin. Kidney Int, 51(4), pp. 1053-8

- Lippi, I., Guidi, G., Marchetti, V., Tognetti, R. & Meucci, V. (2014). Prognostic role of the product of serum calcium and phosphorus concentrations in dogs with chronic kidney disease: 31 cases (2008-2010). J Am Vet Med Assoc, 245(10), pp. 1135-40
- Littman, M.P., Wiley, C.A., Raducha, M.G. & Henthorn, P.S. (2013). Glomerulopathy and mutations in NPHS1 and KIRREL2 in soft-coated Wheaten Terrier dogs. Mamm Genome, 24(3-4), pp. 119-26.
- Lund, E.M., Armstrong, P.J. and Kirk, C.A. (1999). Health status and population characteristic of dog and cats examined at private veterinary practice in United States. Journal of American Veterinary Research Association, 214: 1336-1341.
- Lunn KF. The Kidney in critically ill small animals. Veterinary Clinics of North America—Small Animal Practice, v. 41, n. 4, p. 727–744, 2011.
- Maack, T., Johnson, V., Kau, S.T., Figueiredo, J. & Sigulem, D. (1979). Renal filtration, transport, and metabolism of low-molecular- weight proteins: a review. Kidney Int, 16(3), pp. 251-70.
- MacDougall, I.C., Cooper, A.C. (2002). Erythropoietin resistance: the role of inflammation and proinflammatory cytokines. Nephrology Dialysis Transplantation, 17(11):39–43.

- Maddison, J., Syme, H. (2010). Chronic kidney disease in dogs and cats II: Principles of management. Irish Veterinary Journal, 63(2): 106-109
- Manczur, F., Hetyey C., Reczigel J. (2003) Occurance of canine cardiological disease diseases in Hungary (1997-2000). Magy Allatorvosok, 125; 669-682.
- Mimura, I. & Nangaku, M. (2010). The suffocating kidney: tubulointerstitial hypoxia in end-stage renal disease. Nat Rev Nephrol, 6(11), pp. 667-78.
- Mitchell, A.R., Bywater, R.J., Clarke, K. W. (1989). Urinary disorders and fluid therapy. In: Veterinary fluid therapy. Blackwell, Scientific Pub., London. pp: 193-214.
- Morris SM Jr. Arginine metabolism: boundaries of our knowledge. Journal of Nutrition. 2007; 137:1602S–1609S.
- Mortensen, S.A.(1993).Prospective on therapy of cardiovascular diseases with coenzyme Q10(Ubiquinone). Clin. Invest., 71(Suppl 8):S116-S123
- Muir, P., Tilzey, A.J., English, T.A.H., Nicholson., F., Signy, M. and Banatvala, J.E. (1989). Chronic relapsing pericarditis and dilated cardiomyopathy: serological evidence of persistent en-terovirus infection. Lancet., 804–806.

- Munday, J.S.; Dyer, C.B.; Hartman, A. C. and Orbell, G. M. (2006). A possible predisposition to dilated cardiomyopathy in Huntaways dogs. N. Z. Vet. J., 54: 231-4.
- Nyland T.G., Mattoon J.S., Wisner E.R.: Ultrasonography of the urinary tract and Adrenal glands, Upper urinary tract. In: Nyland T.G. e Mattoon J.S.: Veterinary diagnostic ultrasound. Philadelphia: W.B. Saunders Company 1995, pp 95-111.
- Nakamoto H, Fuller SB, Robaczewski DL, Winicov E, Ferrario CM, Dean RH. Effects of chronic nitric oxide inhibition on the evolution of chronic renal ischemia in two-kidney, one-clip hypertension. In: Oliver KC, Mason SK, Lawrence KS, eds. Surgical Forum. Chicago, Ill: American College of Surgeons; 1994:385-388
- Nakamoto H, Fuller SB, Robaczewski DL, Winicov E, Ferrario CM, Dean RH. Effects of chronic nitric oxide inhibition on the evolution of chronic renal ischemia in two-kidney, one-clip hypertension. In: Oliver KC, Mason SK, Lawrence KS, eds. Surgical Forum. Chicago, Ill: American College of Surgeons; 1994:385-388.
- Nath, K.A. (1992). Tubulointerstitial changes as a major determinant in the progression of renal damage. Am J Kidney Dis, 20(1), pp. 1-17.
- Natoli, J.L., Boer, R., Nathanson, B.H., Miller, R.M., Chiroli, S., Goodman, W.G. & Belozeroff, V. (2013). Is there an association between elevated or low serum levels of phosphorus, parathyroid

hormone, and calcium and mortality in patients with end stage renal disease? A meta-analysis. BMC Nephrol, 14, p. 88.

- Nelson R.W., Couto C.G. Small animal internal medicine – fourth edition, 2009, Elsevier
- Nenov, V.D., Taal, M.W., Sakharova, O.V. & Brenner, B.M. (2000). Multi-hit nature of chronic renal disease. Curr Opin Nephrol Hypertens, 9(2), pp. 85-97.
- Nowend, K.L., Starr-Moss, A.N., Lees, G.E., Berridge, B.R., Clubb, F.J., Kashtan, C.E., Nabity, M.B. & Murphy, K.E. (2012). Characterization of the genetic basis for autosomal recessive hereditary nephropathy in the English Springer Spaniel. J Vet Intern Med, 26(2), pp. 294-301.
- O'Grady MR: Acquired valvular heart disease. In Ettinger SJ, Feldman SJ (eds): Textbook of Veterinary Internal Medicine, ed 4. Philadelphia, WB Saunders, 1995, p 944
- Oliver, J.D., 3rd, Anderson, S., Troy, J.L., Brenner, B.M. & Deen,
- W.H. (1992). Determination of glomerular size-selectivity in the normal rat with Ficoll. J Am Soc Nephrol, 3(2), pp. 214-28.
- Onuigbo, M.A. & Agbasi, N. (2014). Chronic kidney disease prediction is an inexact science: The concept of "progressors" and "nonprogressors". World J Nephrol, 3(3), pp. 31-49.

- Orlova T.R.,. Elizarova E.P, Ryff I.M., Fetisova N.I., Mit'kina L.I.:Use of taurine in the treatment of experimental congestive heart failure Kardiologiia, 31 (1991), pp. 77-80
- Paakkari I, Lindsberg P. Nitric oxide in the central nervous system. Ann Med. 1995;27:369–77
- Pedersen, H.D., Olsen, L.H., Mow, T. and Christensen, N.J. (1999).Neuroendocrine changes in Dachshunds with mitral valve prolapse examined under different study conditions. Res. Vet. Sci., 66:11–17.
- Perkovic, LD, Rumboldt, D, Bagatin, Z. (2002). Comparison of ondansetron with metoclopramide in the symptomatic relief of uremia-induced nausea and vomiting. Kidney Blood Press Res, 25: 61-64.
- Peterson, J.C., Adler, S., Burkart, J.M., Greene, T., Hebert, L.A., Hunsicker, L.G., King, A.J., Klahr, S., Massry, S.G. & Seifter, J.L. (1995). Blood pressure control, proteinuria, and the progression of renal disease. The Modification of Diet in Renal Disease Study. Ann Intern Med, 123(10), pp. 754-62.
- Pezza V, Bernardini F, Pezza E, Pezza B, Curione M. Study of supplemental oral l-arginine in hypertensives treated with enalapril + hydrochlorothiazide. Am J Hypertens. 1998 Oct;11(10):1267-70.
- Phang JM, Liu W, Hancock C, et al. The proline regulatory axis and cancer. Front Oncol 2012; 2:60

- Polichnowski, A.J., Lan, R., Geng, H., Griffin, K.A., Venkatachalam, M.A. & Bidani, A.K. (2014). Severe renal mass reduction impairs recovery and promotes fibrosis after AKI. J Am Soc Nephrol, 25(7), pp. 1496-507.
- Polzin D.J., Osborne, C.a., adams l.g. and lulich j.p. (1992), medical management of feline chronic renal failure, current veterinary therapy XI, philadalphia, Pa: W.B. saunders co., pp: 848- 853
- Polzin, 2011 Polzin D.J. Chronic kidney disease. In: Bartges J, Polzin DJ, editors. Nephrology and urology of small animals. Ames (IA): Wiley-Blackwell; 2011. pp. 433-71
- Polzin, D. J. (2011). Chronic kidney disease in small animals. Veterinary Clinics of North America: Small Animal Practice, 41(1): 15-30.
- Polzin, D., Osborne, C., Jacob F., Ross, S. (2000). Chronic renal failure. Textbook of veterinary internal medicine, 2: 1734-1760.
- Polzin, D., Ross, S., Osborne, C., Lulich, J., Swanson, L. (2005). Clinical benefit of calcitriol in canine chronic kidney disease. Journal of Veterinary Internal Medicine, 19: 433.
- Polzin, D.J. (2010). Chronic kidney disease. In: Ettinger SJ, Feldman EC, editors, Textbook of veterinary internal medicine, St Louis (MO): Saunders Elsevier, 2: 1990–2021.

- Polzin, D.J. . Staged Management of Chronic Kidney Disease in Dogs and Cats. World Small Animal Veterinary Association World Congress Proceedings, 2009
- Prasad, K., Mantha, S., Kalra, J. and Lee, P. (1997) Hypercholesterolemiainduced oxidative stress in heart and its prevention by vitamin E. Int. J. Angiol., 6:13–17
- Pyle, R.L., Abbott, J. and MacLean, H. (2004). Pulmonary Hypertension and Cardiovascular Sequelae in 54 Dogs. Intern. J. Appl. Res. Vet. Med., 2(2): 99-109
- Randolph, J. E., Scarlett, J., Stokol, T., Macleod, J. N. (2004). Clinical efficacy and safety of recombinant canine erythropoietin in dogs with anemia of chronic renal failure and dogs with recombinant human erythropoietin-induced red cell aplasia. Journal of Veterinary Internal Medicine, 18: 81-91
- Rang, H.P., Dale, M.M., Ritter, J.M., Flower, R.J. (2007). Drugs affecting major organ system. In: Text book pharmacology, 5.
- Reece W. O.: Functional Anatomy and Physiology of Domestic Animals. Fourth edition 2009 Wiley-Blackwell
- Richardson, P., McKenna, W., Bristow, M., Maisch, B., Mautner, B., O'Connell, J., Olsen, E., Thiene, G., Goodwin, J., Gyarfas, I., Martin, I. and Nordet, P. (1996). Report of the 1995 World Health Organisation/International Society and Federation of Cardiology Task

Force on the definition and classification of cardiomyopathies. Circ., 93: 637–640.

- Robertson, J., Seguin, M. A., (2006). Renal disease- case based approach to acute renal failure and protein losing nephropathy, IDEXX Laboratories, Inc. 4298-01(8).
- Rozario, T. & DeSimone, D.W. (2010). The extracellular matrix in development and morphogenesis: a dynamic view. Dev Biol, 341(1), pp. 126-40
- Schainuck, L.I., Striker, G.E., Cutler, R.E. & Benditt, E.P. (1970). Structural-functional correlations in renal disease. II. The correlations. Hum Pathol, 1(4), pp. 631-41
- Schlondorff, D. (1987). The glomerular mesangial cell: an expanding role for a specialized pericyte. Faseb j, 1(4), pp. 272-81.
- Sebbag,L., Forrat,R., Canet,E., Renaud,S., Delaye,J. and de Lorgeril,M. (1994). Effects of dietary supplementation with alpha- tocopherol on myocardial infarct size and ventricular arrhythmias in a dog model of ischemiareperfusion. J. Am. Coll. Cardiol., 24: 1580- 1585.
- Segev G, Nivy R, Kass PH, Cowgill LD. A retrospective study of acute kidney injury in cats and development of a novel clinical scoring system for predicting outcome for cats managed by hemodialysis.Journal of Veterinary Internal Medicine, v. 27, n. 4, p. 830–839, 2013.

- Segev, G. (2011). Outcome Prediction of Acute Kidney Injury in Dogs and Cats. Israel Journal of Veterinary Medicine, 66(3): 82-88
- Seikrit, C., Henkel, C., van Roeyen, C.R., Bokemeyer, D., Eitner, F., Martin, I.V., Boor, P., Knuchel, R., Meyer, H.E., Muller-Newen, G., Eriksson, U., Floege, J. & Ostendorf, T. (2013). Biological responses to PDGF-AA versus PDGF-CC in renal fibroblasts. Nephrol Dial Transplant, 28(4), pp. 889-900
- Simkhovich, B.Z., Kleinman, M.T. and Kloner, R.A. (2008). Air pollution and cardiovascular injury: Epedemiology, toxicology and mechanism. J. Am. Coll. Cardiol., 52:719-726
- Singer, M.A. (2001). Of mice and men and elephants: metabolic rate sets glomerular filtration rate. Am J Kidney Dis, 37(1), pp. 164-178.
- Sisson D: Acquired valvular heart disease in dogs and cats. In Bonagura JD (ed): Cardiology: Contemporary Issues in Small Animal Practice. New York, Churchill Livingstone, 1987, p 59 75
- Sleeper, M.; Henthorn, P.S.; Vijayasarathy, C.; Dambach, D.M..; Bowers, T.; Tijskens, P.; Armstrong, C.F. and Lankford, E.B. (2002). Dilated cardiomyopathy in juvenile Portuguese Water Dogs. J. Vet. Int. Med., 16: 53-62.
- Stanek, G., Klein, J., Bittner, R. and Glogar, D. (1990). Isolation of Borrelia burgdorferi from myocardium of a patient with longstanding cardiomyopathy. N. Engl. J .Med., 322:249– 252.

- Sukullaya, A., Anuchai, N., (2008). A retrospective study of clinical use of recombinant human erythropoietin for treatment of anemia in dogs with renal failure in Bangkok, Thailand. Comparative Clinical Pathology, 17(3):165-170.
- Sykes, J.E., Kittleson, M.D., Chomel, B.B., Macdonald, K.A. and Pesavento, P.A. (2006). Clinicopathologic findings and outcome in dogs with infective endocarditis: 71 cases (1992–2005). J. Am. Vet. Med. Assoc., 228 (11): 1735-1747.
- Syme, H.M., Markwell, P.J., Pfeiffer, D. & Elliott, J. (2006). Survival of cats with naturally occurring chronic renal failure is related to severity of proteinuria. J Vet Intern Med, 20(3), pp. 528-35
- Tan BE, Yin YL, Liu ZQ, Li XG, Xu HJ, Kong XF, Huang RL, Tang WJ, Shinzato I, Smith SB, Wu GY. Dietary L-arginine supplementation increases muscle gain and reduces body fat mass in growing finishing pigs. Amino Acids. 2009; 37:169–175.
- Tanaka, S., Tanaka, T. & Nangaku, M. (2014). Hypoxia as a key player in the AKI-to-CKD transition. Am J Physiol Renal Physiol, 307(11), pp. F1187-95
- Tapiero H, Mathé G, Couvreur P, Tew KD. L-Arginine. Biomedicine and Pharmacotherapy. 2002;56(9):439-45.
- Tenhündfeld, J., Wefstaedt, P., and Nolte, I. J. (2009). A randomized controlled clinical trial of the use of benazepril and heparin for the

treatment of chronic kidney disease in dogs. Journal of the American Veterinary Medical Association, 234(8): 1031-1037.

- Tidholm, A. (2006). Survival in dogs with dilated cardiomyopathy and congestive heart failure treated with digoxin, furosemide and propranolol: A retrospective study of 62 dogs. J. Vet Cardiol., 8: 41-47.
- Tidholm, A. and Jonsson, L. (1997). A retrospective study of canine dilated cardiomyopathy (189 cases). J. Am. Anim. Hosp. Assoc., 33:544–550.
- Tidholm, A. and Jonsson, L. (2005). Histologic Characterization of Canine Dilated Cardiomyopathy. Vet.Pathol., 42: 1–8.
- Tidholm, A.E.M. (1996): Canine dilated cardiomyopathy: A study of 189 cases in 38 breeds. Vet. Quarterly, 18(1): 34-35.
- Tilley, L.P. (1985). Analysis of P-QRS-T deflections. In: Essentials of canine and feline electrocardiogrphy, 2nd Ed., Lea and Febiger, Philadelphia, USA., pp. 57-97.
- Tilley, L.P. (1985). Essentials of canine and feline electrocardiography. Interpretation and treatment, 2nd Ed., Lea and Febiger, Philadelphia, USA.
- Toda N. 2003. Vasodilating beta-adrenoceptor blockers as cardiovascular therapeutics. Pharmacol Ther, 100:215–34.

- Torres, C. L., Backus, R. C., Fascetti, A. J. and Rogers, Q. R. (2003). Taurine status in normal dogs fed a commercial diet associated with taurine deficiency and dilated cardiomyopathy. J. Anim. Physiol. Anim. Nutr. 87: 359–372.
- Tozawa, M., Iseki, K., Iseki, C., Kinjo, K., Ikemiya, Y. & Takishita, S. (2003). Blood pressure predicts risk of developing end-stage renal disease in men and women. Hypertension, 41(6), pp. 1341-5.
- Trujillo ME, Sullivan S, Harten I, Schneider SH, Greenberg AS, Fried SK. Interleukin-6 regulates human adipose tissue lipid metabolism and leptin production in vitro. Journal of Clinical Endocrinology and Metabolism. 2004; 89:5577–5582.
- Tufani, N.A., Singh, J.L., Kumar, M., Gupta, D., Shekhar, P. and Rajora, V.S. (2015). Renal failure in Indian dogs: An epidemiological study. Indian Journal of Veterinary Medicine, 35(1): 7-11.

- Tufani, N.A., Singh, J.L., Kumar, M., Gupta, D., Shekhar, P. and Rajora, V.S. (2015). Renal failure in Indian dogs: An epidemiological study. Indian Journal of Veterinary Medicine, 35(1): 7-11.

- Uzlu, E., Kalanbacak, A. (2005). Evaluation of secondary hypertension by oscillometric method in dogs with renal failure, symptoms, treatment, with angiotensin II receptor antagonists. Ankara Universitesi Veteriner Fakultesi Dergisi, 52 (1): 33-38.

- Vallance P, Leon A, Calver A, Collier J, Moncada S. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. Lancet 1992; 339: 572-5
- Valocikova, I., Kristofova, B., Valocik, G. (2008). Cardiac biomarkrs and chronic renal diseases. Bratisl. Lek. Listy., 109(8): 341-344.
- Van Fleet, J.F. and Ferrans ,V.J. (1986). Myocardial diseases of animals. Am. J. Pathol., 124: 98–178.
- Venkatachalam, M.A., Griffin, K.A., Lan, R., Geng, H., Saikumar, P. & Bidani, A.K. (2010). Acute kidney injury: a springboard for progression in chronic kidney disease. Am J Physiol Renal Physiol, 298(5), pp. F1078-94
- Venkatachalam, M.A., Weinberg, J.M., Kriz, W. & Bidani, A.K. (2015). Failed Tubule Recovery, AKI-CKD Transition, and Kidney Disease Progression. J Am Soc Nephrol, 26(8), pp. 176576.
- Vilafranca, M., Wohlsein, P. & Trautwein, G. (1995). Expression of class II major histocompatibility complex molecules in renal tubular epithelial cells of canine kidneys affected with tubulointerstitial nephritis. Res Vet Sci, 59(2), pp. 114-7.
- Vitalaru, B.A., Micsa, C. (2015). Peritoneal dialysis in acute renal and liver failure. Agriculture and Agricultural Science Procedia, (6): 392 – 396.

- Ware, W.A. (2003). Common congenital cardiac anomalies. In. Nelson R.W., Couto, C.G. (eds): Small Animal Internal Medicine. 3rd Ed. Mosby, Philadelphia. 151-168.
- Wess,G., Schulze, A., Butz,V., Simak,J., Killich,M., Keller, L.J.M., Maeurer,J. and, Hartmann, K. (2010).Prevalence of Dilated Cardiomyopathy in Doberman Pinschers in various Age Groups. J. Vet. Intern. Med., 24(1): 1-6.
- Westgarth, C., Christley, R. M. & Christian, H. E. How might we increase physical activity through dog walking?: A comprehensive review of dog walking correlates. Int J Behav Nutr Phys Act 11, 83, doi:10.1186/1479-5868-1183 (2014)
- White, J.V., Finco, D.R., Crowell, W.A., Brown, S.A. & Hirakawa, D.A. (1991). Effect of dietary protein on functional, morphologic, and histologic changes of the kidney during compensatory renal growth in dogs. Am J Vet Res, 52(8), pp. 1357-65.
- William O. Reece : Functional Anatomy and Physiology of Domestic Animals. Fourth edition 2009 Wiley-Blackwell
- Williams TL, Elliott J, Syme HM. Measurement of systolic blood pressure (SBP) in cats by the indirect Doppler technique is not altered by the use of headphones. ECVIM Congress 2010.

- Williams, P.S., Fass, G. & Bone, J.M. (1988). Renal pathology and proteinuria determine progression in untreated mild/moderate chronic renal failure. Q J Med, 67(252), pp. 343-54.
- Wright, K. N.; Atkins, C.E. and Kanter, R. (1996). Supraventricular tachycardia in our young dogs. J. Am. Vet. Med. Assoc., 1: 75-80
- Wu G, Lee MJ, Fried SK. The arginine-NO pathway modulates lipolysis in adipose tissues of obese human subjects. FASAB Journal. 2007;21: A1052
- Wu G, Meininger CJ. Nitric oxide and vascular insulin resistance. BioFactors. 2009; 35:21–27.
- Wu G, Morris SM Jr. Arginine metabolism: nitric oxide and beyond. Biochemical Journal. 1998; 336:1–17.
- Wu G. Amino acids: metabolism, functions, and nutrition. Amino Acids. 2009; 37:1–17.
- Xu, G., Luo, K., Liu, H., Huang, T., Fang, X. & Tu, W. (2015). The progress of inflammation and oxidative stress in patients with chronic kidney disease. Ren Fail, 37(1), pp. 45-9.
- Yozova, I. D., Howard, J., and Adamik, K. N. (2016). Retrospective evaluation of the effects of administration of tetrastarch (hydroxyethyl starch 130/0.4) on plasma creatinine concentration in dogs (2010– 2013):

201 dogs. Journal of Veterinary Emergency and Critical Care, 26(4): 568-577

- Yu, S., Gross, K. L., Allen, T. A. (2006). A renal food supplemented with vitamins E, C and betacarotene reduces oxidative stress and improves kidney function in client-owned dogs with stages 2 or 3 kidney disease. Journal of Veterinary Internal Medicine, 20: 1537.
- Zoja, C., Abbate, M. & Remuzzi, G. (2015). Progression of renal injury toward interstitial inflammation and glomerular sclerosis is dependent on abnormal protein filtration. Nephrol Dial Transplant, 30(5), pp. 706-12.