

**Thiamine Concentrations in Extruded Dog and Cat Food & Determination of  
Thiamine Status in Healthy Dogs and Cats and Comparison with Hospitalized  
Inappetent Animals**

by

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## **ABSTRACT**

### **THIAMINE CONCENTRATIONS IN EXTRUDED DOG AND CAT FOOD & DETERMINATION OF THIAMINE STATUS IN HEALTHY DOGS AND CATS AND COMPARISON WITH HOSPITALIZED INAPPETENT ANIMALS**

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Thiamine is a water-soluble vitamin and a dietary requirement for dogs and cats. It has a critical role in energy metabolism. As pet owners may freeze excess pet food in an attempt to maintain freshness, this thesis investigates the effect of freezing on thiamine in extruded dog and cat foods. Storage temperature did not affect thiamine concentrations of extruded diets, but thiamine significantly increased over time. Additionally, this thesis examines thiamine status of healthy dogs and cats in comparison to inappetent patients presenting to a tertiary referral hospital, and determines the effect of supplementation on resultant thiamine status in inappetent dogs. We found that thiamine diphosphate (TDP) and unphosphorylated thiamine status of healthy dogs declined with age. This relationship was not present in cats. TDP status was higher in inappetent dogs compared to healthy dogs. Supplementation led to higher TDP status in inappetent dogs compared to baseline TDP status.

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## LIST OF ABBREVIATIONS

AAFCO – Association of American Feed Control Officials  
Acetyl CoA – acetyl coenzyme A  
ADP – adenosine diphosphate  
AHE – Alaskan husky encephalopathy  
AI – adequate intake  
AMP – adenosine monophosphate  
ATDP – adenosine thiamine diphosphate  
ATP – adenosine triphosphate  
ATTP – adenosine thiamine triphosphate  
BCS – body condition score  
BW – body weight  
CBC – complete blood count  
CF – crude fibre  
CHF – congestive heart failure  
CKD – chronic kidney disease  
CP – crude protein  
CSF – cerebrospinal fluid  
DMB – dry matter basis  
EDTA - Ethylenediaminetetraacetic acid  
EE – ether extract  
ETKA – erythrocyte transketolase activity  
F – intact female  
FEDIAF – European Pet Food Industry Foundation  
FeLV – feline leukemia virus  
FS – spayed female  
GI – gastrointestinal  
HPLC – high-performance liquid chromatography  
ICU – intensive care unit  
IM – intramuscular  
IV – intravenous  
MCS – muscle condition score  
ME – metabolisable energy  
MN – neutered male  
MRI – magnetic resonance imaging  
NADH – nicotinamide adenine dinucleotide  
NRC – National Research Council  
pH – potential of hydrogen  
P<sub>i</sub> – inorganic phosphate  
PP<sub>i</sub> – inorganic pyrophosphate  
RA – recommended allowance  
RBC – red blood cell  
RER – resting energy requirement  
SLC – solute carrier  
SQ – subcutaneous

SUL – safe upper limit  
TCA – trichloroacetic acid  
TDP – thiamine diphosphate  
THTR – thiamine transporter  
TK – transketolase  
TMP – thiamine monophosphate  
TPP – thiamine pyrophosphate  
TTP – thiamine triphosphate  
WSAVA – World Small Animal Veterinary Association

# CHAPTER 1: THE ROLE OF THIAMINE AND THE EFFECTS OF DEFICIENCY IN DOGS AND CATS: A REVIEW

## 1.1 – Historical Importance of Thiamine

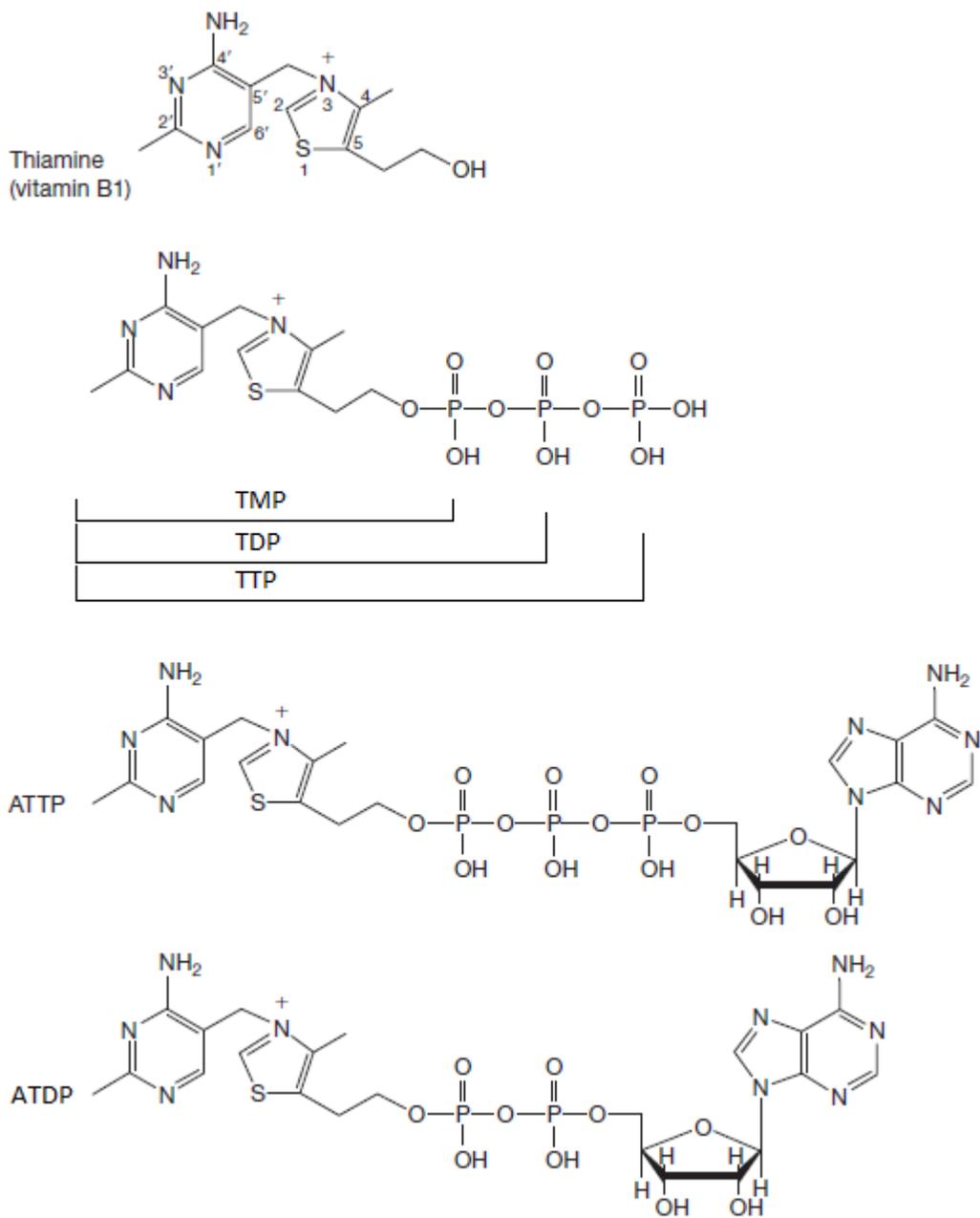
Thiamine was the first of the B vitamins to be discovered, and thus was given the name vitamin B<sub>1</sub> [1]. Thiamine was discovered accidentally when researchers searched for a cure for beriberi, a disorder consisting of neurological and cardiovascular symptoms that can manifest either as a “dry” form that consists of neurological symptoms and muscle wasting, or a “wet” form that has cardiovascular symptoms and is characterized by edema [2]. The disease has been known since the 10<sup>th</sup> century China, where it was originally believed to be a result of inhaling noxious swamp gases [3]. Later beliefs about beriberi included some considerations that it might be associated with malnutrition, as well as hypotheses that the disease was caused by a then-undiscovered micro-organism [3]. Early beriberi research performed by Christian Eijkman in 1890 was conducted by inoculating chickens with blood of humans diagnosed with beriberi. However, clinical signs of a neurological condition that Eijkman called polyneuritis manifested in both the chickens injected with blood of beriberi patients and in the control chickens that had not been injected [4]. When these signs later disappeared in both groups, it was discovered that the chickens’ diets had changed at that time from polished white rice to brown rice [4], suggesting that some aspect of the milling process was involved in the manifestation of the disease. Later research conducted by Gerrit Grijns built on Eijkman’s results and through a series of experiments determined that beriberi was due to a deficiency of nutrients that were not the fats, carbohydrates, or proteins known at the time, and which were later named vitamins [5]. From that research, thiamine itself was discovered and its structure identified [6].

Thiamine deficiency also has a long history in veterinary medicine, with early reports in the 1930s of a disease that appeared on a fox fur farm with clinical signs similar to those of thiamine deficiency in humans [7]. This disease, called Chastek's paralysis due to its emergence on the Chastek fox fur farm, is a nervous system disease with signs including anorexia, cachexia, and depression [8]. Since then, signs of thiamine deficiency have been seen in several other non-human species, including mink [9], dogs [10-12], and cats [12-15].

## **1.2 – Biochemistry and Sources of Thiamine**

### **1.2.1 – Structure and Function of Thiamine and its Phosphate Esters**

Thiamine (3-(4-amino-2-methyl-pyrimidyl-5-methyl)-4-methyl-5-( $\beta$ -hydroxyethyl)thiazole) consists of a thiazole ring and a pyrimidine ring linked by a methylene bridge [2]. It can exist in the blood in a non-phosphorylated form (free thiamine), or it can be phosphorylated at the alcohol side-chain of the thiazole ring to create either thiamine monophosphate (TMP, one phosphate group), thiamine diphosphate (TDP, two phosphate groups), or thiamine triphosphate (TTP, three phosphate groups) [2]. It was recently discovered that thiamine can also exist in an adenylated form, as either adenosine thiamine diphosphate (ATDP) or adenosine thiamine triphosphate (ATTP) [16, 17]. The structures of thiamine and its derivatives are shown in Figure 1.1. Figure 1.2 describes the metabolic pathway of thiamine and its derivatives.



**Figure 1.1.** Structure of thiamine and its phosphate esters.

ATDP – adenosine thiamine diphosphate; ATTP – adenosine thiamine triphosphate; TDP – thiamine diphosphate; TMP – thiamine monophosphate; TTP – thiamine triphosphate. Image adapted from [22].

Cellular concentrations of free thiamine vary widely between mammalian species. Free thiamine can make up approximately 5-20% of cellular thiamine concentrations, though this value can vary between study

populations and whole blood concentrations of free thiamine can be as low as 2% in humans [18-20]. Free thiamine may be found in higher concentrations in plasma and cerebrospinal fluid (CSF) compared to whole blood [19]. However, plasma free thiamine concentrations in another population of humans were comparable to values in whole blood, while red blood cell (RBC) free thiamine concentrations were significantly lower than both plasma and whole blood [20]. Free thiamine can be ingested directly from plant sources [1]. It has a function in synaptic transmission and cell membrane conductance, and stimulates activity in the spinal cord and cerebellum [21].

Thiamine monophosphate is synthesized when TDP dephosphorylates via cytosolic thiamine diphosphatase [22]. It can make up between 4-6% of total thiamine in non-human mammals, and concentrations are similarly low in human whole blood [18-20]. One study found that plasma and whole blood TMP concentrations in humans are comparable, while concentrations in RBCs are lower than those of either plasma or whole blood [20]. However, concentrations of TMP are increased in CSF compared to whole blood [19]. In mammals, TMP is thought to be an inactive form of thiamine that can be transformed again to provide active forms as needed [21].

Thiamine diphosphate, which is also known as thiamine pyrophosphate (TPP), is the main circulating form of thiamine, and makes up approximately 70-90% of the body's thiamine content in mammals and approximately 80% of whole blood thiamine concentrations in humans [18-20]. Most TDP in human blood is found in RBCs, though concentrations in RBCs are lower than those in whole blood and plasma [20]. Thiamine diphosphate can be directly ingested from meat sources, or it can be synthesized by free thiamine that has undergone phosphorylation via cytosolic thiamine diphosphokinase or by TTP that has been dephosphorylated using 25-kDa TTPase [22].

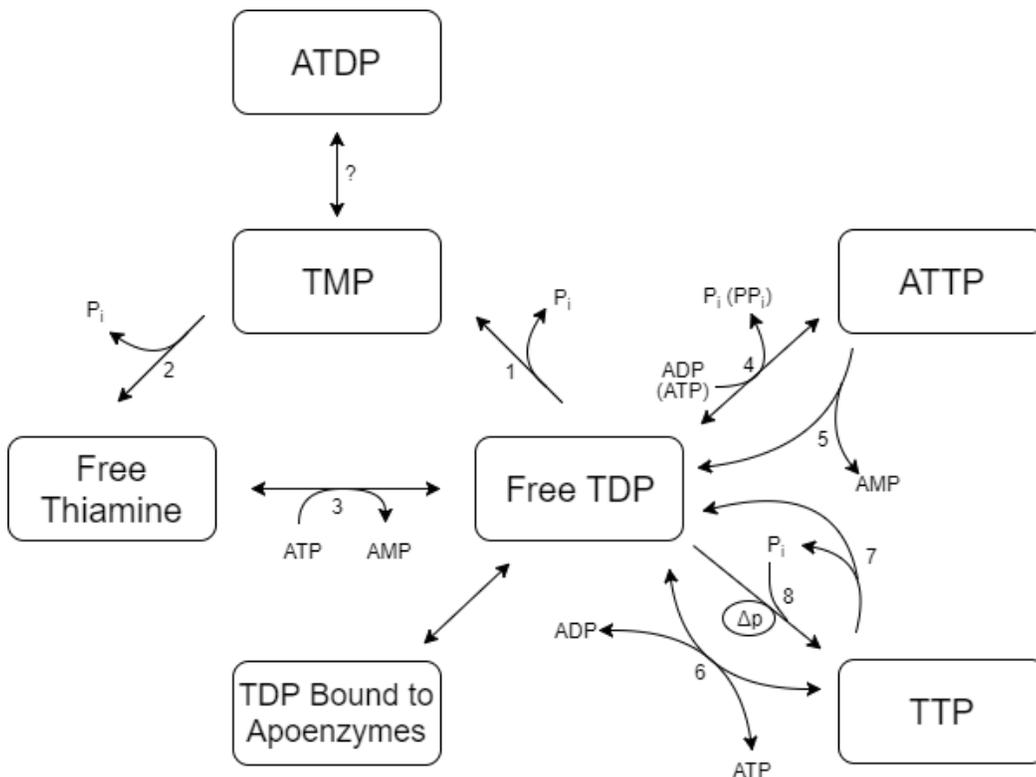
In the cytosol, TDP acts as a cofactor to transketolase, which shunts excess sugars from the pentose phosphate pathway to the glycolytic pathway to be converted to pyruvate [22]. After pyruvate enters the mitochondrion and begins the Krebs cycle, TDP is a cofactor for the pyruvate dehydrogenase-mediated conversion of pyruvate to acetyl co-enzyme A, and later for the enzymatic conversion of  $\alpha$ -ketoglutarate to succinate via  $\alpha$ -ketoglutarate dehydrogenase [1]. It is also a cofactor for the branched-chain  $\alpha$ -keto acid dehydrogenase-mediated conversion of branched-chain  $\alpha$ -keto acids into their respective acyl coenzyme A (acyl CoA) [1]. Thiamine diphosphate's role in the Krebs cycle makes it critical for the eventual production of adenosine triphosphate (ATP), and its function within the pentose phosphate pathway assists with the production of nucleotides and of nicotinamide adenine dinucleotide (NADH), which acts as an electron carrier to produce ATP in the electron transport chain [1, 2].

An adenylated form of TDP, ATDP, has recently been discovered in *Escherichia coli* [17], but it has not presently been found in animal tissues except for almost-undetectable concentrations in quail liver [17]. The function of ATDP remains unknown.

Thiamine triphosphate is created by the cytosolic adenylate kinase-mediated phosphorylation of TDP [22]. It can be found in all body tissues, but is typically present at low concentrations in the body, and generally makes up less than 1% of total body thiamine [18, 22]. Thiamine triphosphate is found in the highest abundance in the brain and skeletal muscles [18, 19], suggesting a role in nervous function and transmission. Thiamine triphosphate may also act as a phosphate donor for proteins in the neuromuscular junction, which suggests that TTP may have a function in cell signaling [23]. Due to its ubiquitous presence throughout the body, TTP may have additional functions outside of the nervous system that have

not yet been discovered [22].

An adenylated form of TTP, named ATTP, was recently discovered in *E. coli*, and is thought to be formed as a response to carbon starvation in bacteria [16]. In rats, it has been found in larger concentrations in the heart, liver, kidney and lung, and at low concentrations in skeletal muscle and brain tissue [16]. Its function remains unclear, though ATTP may act as a storage form of TTP based on initial research in *E. coli* which found that ATTP concentrations diminish and TTP concentrations increase when carbon starvation is reversed [16].



**Figure 1.2.** Interconversions of thiamine and its phosphate esters in human cells. 1 – thiamine diphosphatase (cytosolic); 2 – thiamine monophosphatase (nonspecific); 3 – thiamine diphosphokinase (cytosolic); 4 – TDP adenylyl transferase (cytosolic); 5 – ATTP hydrolase (hypothetical); 6 – adenylate kinase (cytosolic); 7 – 25-kDa TTPase (cytosolic); 8 – TTP synthase (mitochondrial). The mechanism of ATDP metabolism is unknown.

ADP – adenosine diphosphate; AMP – adenosine monophosphate; ATDP – adenosine thiamine diphosphate; ATP – adenosine triphosphate; ATTP – adenosine thiamine triphosphate; P<sub>i</sub> – inorganic phosphate; PP<sub>i</sub> – inorganic pyrophosphate; TDP – thiamine diphosphate; TMP – thiamine monophosphate; TTP – thiamine triphosphate; Δp – transmembrane H<sup>+</sup> gradient. Image adapted from [19, 22].

### 1.2.2 – Sources of Thiamine

Thiamine and its moieties can be synthesized by many species of bacteria, yeast, and plants [24]. During thiamine biosynthesis, a complex biochemical process leads to the synthesis of both the pyrimidine moiety and thiazole moiety, which are then joined together to form a thiamine molecule [24]. Thiamine that has been catabolized into its component parts can also be synthesized in plants. This is achieved through a salvage pathway that re-uses the pyrimidine created during thiamine degradation in order to create more thiamine [24].

Some research suggests that bacteria in the gastrointestinal tract of rats may synthesize thiamine [25], but research in this area has been sparse and the bioavailability of this fecal thiamine has not been studied. Additionally, doses of thiamine instilled into the human large intestine may not lead to a noticeable increase in circulating thiamine concentrations [26], suggesting that intestinal synthesis of thiamine by gastrointestinal bacteria may not be of significant metabolic relevance. Indeed, thiamine is an essential dietary nutrient in several species of mammals, including dogs and cats, as poor thiamine consumption can lead to deficiency and associated diseases [1, 27]. Concentrations of thiamine in foods may vary, though high concentrations of thiamine can be found in foods such as wheat germ, legumes, and pork [28]. Synthetic sources of thiamine, such as thiamine hydrochloride and thiamine mononitrate, can be utilized to fortify foods with thiamine or can be used as supplements to reach optimal thiamine intake requirements [29]. These supplements can be taken orally or can be injected intramuscularly, subcutaneously, or intravenously, though the latter is not recommended due to the risk of adverse reactions [27, 30].

### 1.3 – Dietary Thiamine Absorption and Metabolism

Dietary thiamine is primarily absorbed in the form of free thiamine, due to enzymatic hydrolysis in the gastrointestinal tract by phosphatases that dephosphorylate thiamine's phosphate esters [31-33]. Most dietary thiamine is absorbed through the small intestine [34]. Thiamine is absorbed via active transport if dietary thiamine concentrations are low, while high dietary thiamine concentrations lead to absorption by passive diffusion [32]. Once thiamine enters the intestinal mucosa, it is quickly phosphorylated to one of its phosphate esters [35], but is dephosphorylated again during passage to serosal cells [32]. This dephosphorylated thiamine appears in the plasma as free thiamine, which may then be bound to a protein carrier and transported in RBCs throughout the body, where it is transformed to TDP by thiamine diphosphokinase [22].

Intestinal absorption of thiamine is carrier-mediated, and is regulated by two thiamine transporters (THTR), THTR-1 and THTR-2, which are produced by solute carrier 19 (*SLC19*) genes *SLC19A2* and *SLC19A3*, respectively [36]. THTR-2 has also been found in renal tissue, suggesting that the kidney has a role in re-uptake of circulating thiamine prior to excretion [36]. Excretion of thiamine primarily occurs through urine, though rates of excretion tend to decrease quickly and drastically with suboptimal thiamine ingestion [37]. The kidney is therefore likely to be the primary regulator of thiamine homeostasis in the body [36, 38].

Thiamine is not stored in significant concentrations in the body regardless of whether an individual's thiamine intake is adequate or deficient. This was demonstrated in rats, as similar concentrations of

thiamine were found in organ tissues despite rats receiving either adequate dietary thiamine or no dietary thiamine [39]. Excess dietary thiamine is excreted rather than sequestered in tissues. The liver may act as a storage site for thiamine due to its higher thiamine concentrations compared to other organs, but the overall capacity for thiamine storage is likely low [22].

## **1.4 – Dietary Thiamine in Dogs and Cats**

### **1.4.1 – Thiamine Requirements in Adult Dogs and Cats**

Dogs and cats both require thiamine as part of their diet, but cats require approximately four times more thiamine per day than dogs [27]. This is based on research where thiamine deficiency was induced through feeding of a thiamine-deficient diet. Underlying differences in metabolism between cats and dogs are currently unknown.

Presently, dietary thiamine recommendations have been determined for dogs and cats from case reports and studies measuring varying rates of thiamine intake and resultant health status in each species. In North America, many commercial pet food manufacturers follow Association of American Feed Control Officials (AAFCO) guidelines when formulating diets, which historically are based on recommendations provided by the National Research Council (NRC). The NRC has two sets of dietary thiamine requirements for dogs and cats: adequate intake (AI) recommendations that delineate the minimum thiamine concentration that dogs and cats should receive, as well as the recommended allowance (RA) for thiamine intake. There is currently no safe upper limit (SUL) for thiamine [40]. Because NRC recommendations have historically been based on diets containing highly purified ingredients with high digestibility and nutrient availability [41], AAFCO developed its own recommendations for nutrient intake in more conventional canine and feline diets. Previous AAFCO recommendations for thiamine concentrations of adult dog and cat diets were lower than those set by NRC [42], though these recommendations were updated in 2016 to match the values recommended by the NRC [43]. In Europe, the Fédération Européenne de l'Industrie des Aliments Pour Animaux Familiars (FEDIAF; European Pet Food Industry Federation) has its own set of thiamine recommendations, which are based on the NRC's AI recommendations for adult dogs and cats [44].

According to AAFCO, diets formulated for dogs and cats of all life-stages are recommended to contain at least 2.25 and 5.6 mg thiamine per kg on dry matter basis (DMB), or 0.56 and 1.40 mg/1000 kcal of metabolisable energy (ME) on a calorie basis, respectively [43]. In 2016, these guidelines have been updated from a recommended daily thiamine intake of 1.0 and 5.0 mg/kg on DMB, or 0.29 and 1.25 mg/1000 kcal ME on a calorie basis for dogs and cats, respectively [42]. This update was needed to align with the NRC RA of 2.25 and 5.6 mg thiamine per kg DMB, and 0.56 and 1.40 mg/1000 kcal ME on a calorie basis for dogs and cats, respectively [40]. In Europe, according to the FEDIAF, the RA for thiamine intake is 2.1-2.5 mg/kg DMB or 0.54-0.62 mg/1000kcal ME for dogs, and 4.4-5.9 mg/kg DMB or 1.1-1.47 mg/1000 kcal ME for cats [44]. The recommendations for dietary thiamine concentrations in adult dogs and cats according to the NRC, AAFCO, and FEDIAF are provided in Table 1.1.

**Table 1.1.** Recommendations for thiamine set by the NRC (2006), AAFCO (2017), and FEDIAF (2017) for adult dogs and cats.

			Minimum Adequate Intake	Minimum Recommended Allowance
NRC [40]	DM Basis <sup>a</sup> (mg/kg DMB)	Cats	4.4	5.6
		Dogs	1.8	2.25
	Caloric Basis (mg/1000 kcal ME)	Cats	1.1	1.4
		Dogs	0.45	0.56
	Metabolic Body Weight	Cats <sup>b</sup> (mg/kg <sup>0.67</sup> )	0.11	0.14
		Dogs (mg/kg <sup>0.75</sup> )	0.059	0.074
AAFCO [43]	DM Basis <sup>a</sup> (mg/kg DMB)	Cats	--	5.6
		Dogs	--	2.25
	Caloric Basis (mg/1000 kcal ME)	Cats	--	1.4
		Dogs	--	0.56
FEDIAF [44]	DM Basis <sup>a</sup> (mg/kg DMB)	Cats	--	4.4 <sup>c</sup> 5.9 <sup>d</sup>
		Dogs	--	2.1 <sup>e</sup> 2.5 <sup>f</sup>
	Caloric Basis (mg/1000 kcal ME)	Cats	--	1.10 <sup>c</sup> 1.47 <sup>d</sup>
		Dogs	--	0.54 <sup>e</sup> 0.62 <sup>f</sup>

AAFCO – Association of American Feed Control Officials; FEDIAF – Fédération Européenne de l’Industrie des Aliments Pour Animaux Familiers (European Pet Food Industry Federation); NRC – National Research Council; DMB – dry matter basis; ME – metabolisable energy

<sup>a</sup>Based on a dietary energy density of 4000 kcal ME/kg

<sup>b</sup>Based on a lean cat with an energy intake of 100 kcal x BW<sup>0.67</sup>

<sup>c</sup>Based on a cat with a daily energy requirement of 100 kcal/kg<sup>0.67</sup>

<sup>d</sup>Based on a cat with a daily energy requirement of 75 kcal/kg<sup>0.67</sup>

<sup>e</sup>Based on a dog with a daily energy requirement of 110 kcal ME/kg<sup>0.75</sup>

<sup>f</sup>Based on a dog with a daily energy requirement of 95 kcal ME/kg<sup>0.75</sup>

### **1.4.2 – Analysis of Dietary Thiamine**

Thiamine concentration in food products may be assessed using high-performance liquid chromatography (HPLC) that leads to conversion of thiamine to a fluorescent form known as thiochrome for analysis [45-47]. Microbial methods, which are described in more detail below, have also been described for thiamine analysis in dried yeast [48]. However, thiamine has proven difficult to analyze in food products in the past. One study in 2000 showed that the same HPLC methodologies utilized at different laboratories can lead to fluctuations in reported thiamine concentrations of the same food product, though the authors found that certain methods led to lower variation between results compared to others [49]. The authors suggested that further optimization of a method to analyze thiamine would be beneficial.

In North America, AOAC International (formerly the Association of Official Analytical Chemists) evaluates and publishes analytical methods for a wide range of compounds, including nutrients, pharmaceuticals, and toxins. These methods are then utilized by laboratories to conduct the respective analyses. Currently, AOAC International has published a fluorometric method that uses measurements of thiochrome fluorescence to assess thiamine concentrations in human and pet foods [50].

## **1.5 – Thiamine Status in Healthy Dogs and Cats**

Several methods exist to determine thiamine status. Some are indirect methods that assess thiamine status, such as by measuring the activity of enzymes for which thiamine is a cofactor, while others directly quantify concentrations of thiamine or its phosphate esters.

### **1.5.1 – Indirect Methods to Assess Thiamine Status**

Thiamine status can be estimated using functional tests that measure the activity of enzymes for which thiamine is a cofactor to determine thiamine status. These tests include erythrocyte transketolase activity (ETKA) and the increase in transketolase (TK) activation that is measured after addition of TDP in vitro [33, 51-53]. The percent increase in transketolase activation after TDP addition, where a percentage of >15% correlates to poor thiamine status and >25% is indicative of thiamine deficiency [33], is sometimes referred to as the TDP or TPP effect.

These indirect tests are not without limitations, as results may be impacted by physiological and technical factors. Thiamine deficiency may lead to a decreased amount of apoenzymes capable of being activated by TDP [54], and ETKA in humans also tends to decline with age [55]. Several isoenzymes of transketolase have been identified in human RBCs [56, 57], which could confound analyses of transketolase activation due to potential heterogeneous affinities for TDP [56]. However, the methodology used to identify the number of isoenzymes in human erythrocytes has been challenged [57]. Additionally, freezing (-20°C), refrigeration (4°C), and storage at room temperature can lead to loss of apoenzyme over time and can lead

to thiamine-deficient individuals having a falsely low TDP effect [54].

Thiamine diphosphate is a cofactor for pyruvate dehydrogenase, which converts pyruvate to acetyl co-enzyme A (acetyl CoA) as part of aerobic metabolism. In the absence of adequate enzyme, pyruvate is instead converted to lactate via lactic acidosis (i.e., anaerobic metabolism) [33]. Elevated blood lactate concentrations can therefore be used as indicators of potential thiamine deficiency, and in these cases, thiamine supplementation reverses this lactic acidosis [58, 59].

Thiamine status can also be inferred from assessment of urinary thiamine concentrations, where urinary thiamine concentrations are measured per unit of creatinine to account for renal function [60-62]. A significant correlation is present between thiamine intake and urinary thiamine excretion in adult humans [62]. However, excretion rates can vary widely between individuals, therefore analysis of urinary thiamine may not be an effective method to estimate thiamine intake [62]. In humans, urinary thiamine excretion may also be impacted by some medications (e.g., diuretics), diseases, and dietary carbohydrate concentrations [60, 61, 63, 64]. In dogs and cats, urinary concentrations of organic acids related to dehydrogenase enzyme activity (e.g., lactic acid, 2-hydroxyisovaleric acid, 2-hydroxyadipic acid) can be used as a measure of thiamine status, whereby high concentrations of these compounds can be linked to thiamine deficiency [65, 66].

Measurement of growth response of certain microbial or protozoal species (e.g., yeast, algae) that require thiamine can also be used to determine thiamine status, although these methods are not commonly used for thiamine analysis [67]. Microbial methods have previously been used to assess whole blood thiamine status

in dogs and cats [68, 69].

### **1.5.2 – Direct Methods to Assess Thiamine Status**

Currently, the preferred method of thiamine analysis is to use high-performance liquid chromatography (HPLC) to determine TDP concentrations in RBCs [70]. This method is preferable as it directly measures thiamine concentrations in blood and is therefore not impacted by the physiological processes that can impact results of indirect analyses. Additionally, TDP is stable in whole blood for up to 48h at room temperature, and TDP is stable in hemolysates and whole blood at -70°C for at least 7 months [70]. Red blood cell thiamine concentrations obtained by HPLC are correlated with concentrations in whole blood [70], and are also correlated with results of transketolase activation assays and TDP effect [70, 71].

### **1.5.3 – Thiamine Reference Range in Dogs and Cats**

Little is known about blood thiamine status in dogs and cats. Thus, more research is required to determine an accurate reference range for thiamine in both species.

A small study using microbial methods to compare whole blood thiamine status in one thiamine-deficient dog and six control dogs reported lower thiamine status in the thiamine-deficient animal compared to controls [69], with control dogs having a mean thiamine status of 95 µg/L (range: 84-104 µg/L). However, diets were not nutritionally balanced, and small sample sizes mean that results cannot be generalized

among canine populations. Another study determined whole blood thiamine concentrations in 25 dogs and 29 cats, and found a median blood thiamine concentration of 72  $\mu\text{g/L}$  (range: 46-112  $\mu\text{g/L}$ ) in dogs, and a median of 48  $\mu\text{g/L}$  (range: 20-90  $\mu\text{g/L}$ ) in cats [68]. However, there were methodological concerns in this study. Dogs and cats in this study included growing animals, adults, and senior animals. In addition, how the animals' health status was determined was not clarified and dietary intake was not assessed. This study also used protozoal methods to assess thiamine status by measuring metabolically active forms of vitamins, and while the authors stated that the method had been validated in humans and rats, it was not validated in dogs and cats.

A later study revisited the subject of canine thiamine status, and determined TDP status of whole blood in 64 adult dogs using HPLC. The dogs had a mean  $\pm$  s.d. baseline TDP concentration of  $89.7 \pm 20.7 \mu\text{g/L}$ , which represented a thiamine status of dogs being fed a variety of diets [72]. The authors later put the dogs on one of four diets that each contained known thiamine contents for four months, and found that thiamine status did not differ significantly from the baseline value for any of the diets. However, dietary thiamine intake was not reported. Additionally, thiamine concentrations in this study were not adjusted based on hematocrits when conducting thiamine analyses. While the relationship between thiamine status and hematocrit has not previously been examined, differences in hematocrit between blood samples could affect resultant blood thiamine concentrations due to TDP occurring predominantly within RBCs [33].

## **1.6 – Thiamine Deficiency in Dogs and Cats**

### **1.6.1 – Prevalence of Thiamine Deficiency**

The prevalence of thiamine deficiency in dogs and cats, both healthy and hospitalized, is currently unknown. Thiamine deficiency in elderly people in the United Kingdom has been reported to occur in 8-31% for non-institutionalized individuals and between 23-40% in individuals in nursing homes [73].

Human studies typically examine the prevalence of thiamine deficiency in specific diseases. When compared to the thiamine status of hospitalized control subjects, the prevalence of poor thiamine status has been found to be elevated in individuals with diabetes mellitus (type 1 and 2) [64, 74], congestive heart failure [61], and in alcoholics [75]. However, comorbidities, use of certain medications, diet, and age can impact thiamine status and should be considered as potential factors influencing results in these populations.

One study found that 10% of human ICU inpatients with septicemia are thiamine deficient upon presentation, which increased to 20% within 72h of admission [76]. Thiamine deficiency has been reported in 33-91% of hospital patients with heart disease [77]. Overall, approximately 20% of adult ICU inpatients are thiamine deficient upon admission [78].

## **1.6.2 – Risk Factors and Etiology of Thiamine Deficiency**

### **1.6.2.1 – Inadequate Dietary Thiamine Intake**

In the United States and Canada, over 90% of dogs receive commercial dry or wet dog foods as at least 50% of their diet, and over 85% of dogs receive at least 75% of their diet from commercial foods [79, 80]. Also, cat owners have been found to be more likely to feed commercial pet foods than dog owners, based on a survey of owners in the United States [80]. In North America, commercial cat foods make up at least 50% of the diet for more than 98% of cats, and at least 75% of the diet for over 95% of cats [79, 80]. Since dogs and cats tend to receive most of their nutrition from commercial diets, it is imperative that commercial diets contain adequate concentrations of essential dietary nutrients, including thiamine.

#### **a. Unconventional and Alternative Diets**

Unconventional and alternative diets are diets other than commercial kibble or wet foods, and can include homemade raw and cooked diets, as well as commercially-produced raw diets and diets that resemble homemade diets or that require some preparation from owners (e.g., addition of water or meat) [81]. These types of diets are a growing trend in North America, as owner concerns or suspicions about the nutritional quality of commercial pet foods increase [82].

The majority of homemade diets are likely not nutritionally adequate, with a recent study finding that 95% of homemade diets recipes do not meet nutritional requirements for at least one nutrient, and 83.5% having multiple deficiencies [83]. There have been reports of dogs and cats developing signs of nutrient deficiencies, including thiamine deficiency, as a result of being fed unbalanced homemade diets [10, 14,

69, 84, 85]. Nutritional analyses have shown that homemade diets tend to lack essential macro- and micro-nutrients both when they are intended for adult maintenance [83, 86-88], and when they are intended for the treatment or management of specific diseases [89, 90]. Nutrient imbalances have also been seen in homemade and commercial raw diets, though thiamine content has not specifically been assessed [91-93]. Canine diets formulated by veterinarians often do not meet nutritional requirements for dogs [83]. Thus, homemade diets should be formulated by board-certified veterinary nutritionists to ensure nutritional adequacy. Though, even when formulated to meet nutritional adequacy recommendations, there may be variability in the exact nutritional composition of the ingredients and homemade diet recipe [94]. This makes it difficult to keep the nutrient content of homemade diets consistent between batches. There are also concerns with owners not closely following the recipe, as owners may switch ingredients or alter the preparatory methods of the recipe without consulting a veterinarian or nutritionist, which can lead to unbalancing the diets [94, 95].

#### **b. High Processing Losses During Production of Conventional Pet Food**

In addition to homemade diets, conventional dry and wet diets may contain inadequate thiamine concentrations, as thiamine is very labile and readily degrades under different conditions. This can pose problems for ensuring that commercial diets meet requirements for thiamine concentrations. Thiamine is sensitive to pH, and tends to degrade faster in solutions of neutral and basic pH than in acidic solutions [96, 97], though it tends to be stable in acidic environments [96]. Oxidation will also lead to thiamine degradation [1]. Thiamine will also degrade both when stored in high heat (>120°C) [98] and when stored at ambient temperatures of 25°C [96], though the rate of degradation increases as temperature increases [45]. In pelleted and extruded ruminant feed, thiamine hydrochloride and mononitrate degradation is

approximately 4.0% and 4.5% per month, respectively [99].

Many studies examine thiamine degradation in a buffer solution, thus degradation in food products may show different relationships. Cooking of thiamine-containing foods has variable losses of thiamine, as the exact cooking procedure will lead to different percentages of loss of thiamine [1]. The effect of freezing on thiamine retention is currently unknown. Studies typically examine freezing in addition to another form of processing (e.g., cooking, blanching, canning) [46, 100-102], making results difficult to interpret. One study found that frozen spinach contained higher thiamine content after storage compared to baseline values, but the authors were unable to determine a reason for this result [47].

Pet food manufacturers may compensate for anticipated thiamine degradation by adding additional synthetic thiamine prior to cooking (i.e., extrusion or baking for dry food and canning or cooking for wet food). Despite this, the completed products may not contain adequate quantities of thiamine. Differences in manufacturing procedures between factories and between specific diets may differentially affect thiamine losses, making it difficult to estimate general losses across all dry or wet diets. In general, higher cooking temperatures during extrusion can lead to greater losses of thiamine [103]. However, a recent review examining effects of extrusion on dry pet foods also notes that while higher temperatures may lead to greater thiamine losses, different retention times during extrusion can also lead to variability in thiamine losses between diets [104].

Canned foods seem to be especially susceptible to thiamine deficiency, based on pet food recall information. Since 2010, six recalls of commercial cat foods have occurred for suspected or confirmed

thiamine deficiency in North America, most of which were for canned cat foods. These diets all had AAFCO nutritional adequacy claims. These recalls have consisted of 17 commercial canned cat foods, compared to five extruded cat foods and one raw cat food [105]. The Food Standards Agency (FSA) in the United Kingdom has also reported a recent thiamine-related recall for three dry extruded cat foods [106], and more recently, there has been a recall of eight canned cat foods in Australia that have been found to contain insufficient thiamine [107]. A recent study examining thiamine concentrations in samples of 90 commercial canned cat foods in the United States, all of which claimed to meet AAFCO guidelines, found that 12 of these foods (13.3%) were thiamine-deficient compared to AAFCO (2014) recommended concentrations, and 14 foods (15.6%) were deficient compared to the slightly-higher NRC recommended concentrations [108]. With the 2016 increase in AAFCO recommended concentrations to match those of the NRC, the number of diets in this study that were deficient according to AAFCO recommendations would have been comparable to the NRC (15.6%). The authors of this study also found that paté foods and foods produced by small manufacturing companies had a higher proportion of low thiamine concentrations than non-paté foods and foods produced by large manufacturing companies, respectively. The authors speculated that these differences may have been due to thiamine degradation as a result of the extra processing required to produce paté foods, as well as the ability of larger manufacturers to exert better quality control and conduct more frequent testing for thiamine. The cross-sectional nature of this study, as well as the possibility of inter-batch variation between products and the absence of random selection from all commercially available products, may have impacted results. In contrast, a study that reported thiamine retention after 18 months of storage found 0% thiamine losses in canned dog and cat food over 18 months of storage, but reported storage-related losses of 34.2% and 57.5% in dry dog and cat food, respectively [109]. However, the original research could not be consulted as this research is no longer archived at its publisher, and storage conditions of the diets are therefore unknown.

### **c. Presence of Anti-Thiamine Compounds**

Anti-thiamine compounds, or thiamine agonists, are compounds that inactivate thiamine by changing thiamine's chemical structure. These compounds can be either natural or synthetic.

Thiamine is unique in that it is the sole vitamin for which an enzyme has evolved that can destroy it. This enzyme, thiaminase, is present in the flesh of dead fish and in some shellfish and bacterial species. Thiaminase occurs as two types: type I and type II. Type I thiaminase (EC 2.5.1.2) is a pyrimidine transferase that replaces thiamine's thiazole moiety with other organic compounds, while type II thiaminase (EC 3.5.99.2) is a hydrolase that uses only water as the nucleophile to replace thiazole [1, 110-113]. Thiamine deficiency has been documented in animals that have been fed diets containing predominantly raw fish, including dogs and foxes [7, 10]. Enzymatic thiaminases present in fish are denatured by high heat, with varying temperature sensitivity [114, 115]. Therefore, the risk for thiamine deficiency may be present in both raw and cooked fish-based diets for dogs and cats. These diets may benefit from adding additional supplemental thiamine during processing or as an oral supplement during feeding.

Additionally, the use of sulfites such as sulfur dioxide to preserve meats has been implicated in thiamine loss in foods. There have been documented cases of dogs and cats developing signs of thiamine deficiency after eating sulfite-preserved meat as a primary component of their diet [11, 12, 15]. This is likely due to sulfur's actions in converting thiamine to thiamine disulfide, as this form of thiamine has poor bioavailability in the body [1]. This thiamine-destroying effect occurs in non-meat products as well, where using sulfites such as sodium disulfide during processing can lead to poorer thiamine contents in parboiled

rice [116]. In commercial pet foods, the use of sulfur as a preservative could lead to the loss of bioavailable thiamine and subsequent thiamine deficiency in pets when fed these commercial diets as the primary source of nutrition. Presently, sulfites are not permitted in foods containing meat or sources of thiamine by the United States Food and Drug Administration [117], and AAFCO has similar restrictions on the usage of sulfur and sulfites [43]. This decreases the likelihood of thiamine deficiency caused by sulfites in pets in North America.

The presence of plant-derived anti-thiamine factors has been suspected since 1946, when a study in rats found that clinical signs of thiamine deficiency manifested when rats ate diets where 40% of the daily ration consisted of a species of bracken fern (*Pteris aquilina*) [118]. However, deficiency did not occur in rats that received thiamine supplementation along with the fern-containing diets, and all but one of the rats that developed signs of deficiency recovered after a thiamine supplement was administered, which led the authors to conclude that the fern contained a then-unknown compound with anti-thiamine activity [118]. Unfortunately, this specific study did not specify details about the study population or the composition of the diets. Later research confirmed the existence of plant-derived anti-thiamine factors, as heat-stable compounds known as polyhydroxyphenols, which include caffeic acid, phenols, flavonoids, and tannins, are present in certain plants and destroy thiamine by an oxidative process that transforms it to non-absorbable thiamine disulfide [1, 33, 97, 119-122]. Plants containing polyhydroxyphenols include coffee, tea, and some fruits and vegetables, such as blueberries and red cabbage [33]. While research in dogs and cats has not been conducted to determine the effect of plant-derived anti-thiamine factors on blood nutrient concentrations, the presence of anti-thiamine factors in plant matter may be of importance for dogs and cats being fed home-made diets or large portions of table scraps containing ingredients with these compounds.

#### **d. Interactions with Other Nutrients**

Little has been documented about the interactions of thiamine with other nutrients. A study in kittens showed that high concentrations of dietary glutamic acid led to increased dietary thiamine requirements [123]. The reason for this increased requirement is unknown. Conversely, dietary protein and fat have been found to reduce the metabolic demand for thiamine when compared to carbohydrates [39]. This is likely due to the role of thiamine as a cofactor in carbohydrate metabolism and the increased requirement for thiamine with activation of the Krebs cycle for the catabolism of dietary carbohydrates. The relationship between thiamine status and carbohydrate intake was investigated in adult humans, assessing thiamine status via thiamine concentrations of plasma, urine, and feces, as well as ETKA. Increasing one's consumption of carbohydrates but maintaining a constant thiamine intake led to significant declines in thiamine content when examining plasma and urinary thiamine concentrations. Conversely, there was no difference between altered levels of carbohydrate intake when examining ETKA or fecal thiamine content [60]. However, the study contained only a four-day adaptation period followed by two consecutive phases of increasing carbohydrate intake that each only lasted four days. This short study duration may not have provided enough time for participants to adjust to the diets. Research determining the relationship between carbohydrate intake and thiamine requirements has not been conducted in dogs and cats. This is an area that warrants further research to better delineate thiamine requirements of complete and balanced commercial dog and cat foods that may contain varying carbohydrate concentrations.

### **e. Inadequate Food Intake**

While insufficient dietary thiamine concentrations can be a risk factor for deficiency in dogs and cats ingesting their daily caloric requirements, decreased food intake (hyporexia) or complete lack of food intake (anorexia) can also increase the risk. In these cases, the daily recommended intake of thiamine that is necessary to meet the body's consistent requirement for thiamine is not ingested.

### **f. Life Stage Requirements**

Changes in nutrient requirements can lead to thiamine deficiency if demand for thiamine cofactors increases but intake remains the same. One such scenario is increased demand due to pregnancy and lactation [52, 124], which may lead to higher thiamine requirements due to prolactin's role in regulating the pentose phosphate pathway [124]. In cats, the NRC's RA for thiamine intake currently recommends that queens in gestation and lactation receive higher dietary thiamine concentrations compared to adult maintenance requirements, but recommendations for dogs are identical between both life stages [40]. In contrast, AAFCO's canine and feline recommendations for thiamine are not different between life stages [43]. Higher dietary thiamine concentrations may also be required with increasing activity levels, but research in this area is currently sparse [125].

Elderly individuals may be at higher risk of developing thiamine deficiency, as thiamine absorption is thought to become less efficient as humans age. Additionally, ETKA declines with age [55]. Thiamine status, using ETKA, therefore tends to decline with age [53, 55]. However, analysis of RBCs by HPLC

may show no difference between elderly and young adult groups [53], though other research shows an age-related decline in TDP status [126]. Elderly individuals may have lower thiamine intakes than young adults, but intake may still meet nutrient recommendations for adult humans [53]. As few studies assessed dietary thiamine intake in relation to thiamine status, it is unclear if elderly humans have low thiamine status due to lower thiamine intake, as a result of age-related changes in absorption, or due to comorbidity. More research is therefore required to determine the effect of aging on thiamine metabolism in both humans and companion animals.

#### **1.6.2.2 – Inadequate Thiamine Uptake**

Dogs and cats may develop signs of thiamine deficiency despite ingesting nutritionally adequate concentrations of thiamine, which may be due to several factors affecting the uptake or metabolism of thiamine once it enters the body.

One such factor is intestinal malabsorption or maldigestion, which may occur with chronic GI diseases. More specifically, in humans, inflammatory bowel disease and gastric cancer have been found to be associated with symptoms of thiamine deficiency [127, 128]. However, whether this association is due to insufficient uptake of dietary thiamine from the intestinal lumen or whether this is due to chronic vomiting and diarrhea is currently unknown. Thiamine deficiency can occur as a result of chronic vomiting as thiamine is unable to travel through the GI tract for absorption [129]. Also, chronic diarrhea can lead to poor intestinal uptake of thiamine.

Defects in thiamine transporters THTR-1 and THTR-2 may also be associated with thiamine deficiency. In cats, feline leukemia virus (FeLV), specifically the virus form known as subgroup A (FeLV-A), uses THTR-1 as a receptor to enter cells, which subsequently prevents thiamine uptake through these receptors [130, 131]. However, due to THTR-2 not being affected by this mechanism, the risk of developing thiamine deficiency as a result of FeLV may be low. In dogs, Alaskan Husky Encephalopathy (AHE) is a generally fatal neurological disease that manifests in juvenile Alaskan Huskies, and the genomes of affected dogs have been found to contain a mutation in the gene that produces THTR-2, SLC19A3 [132, 133]. The SLC19A3 gene in dogs has two paralogs, SLC19A3.1 and SLC19A3.2. In the central nervous system of normal dogs, SLC19A3.1 is expressed at high levels and SLC19A3.2 is expressed at low levels. However, dogs with AHE have a novel defect in SLC19A3.1 that has been found to lead to poor expression of this gene [133, 134]. This suggests that the clinical signs of this disease are related to thiamine deficiency of nervous tissues due to an inability of the poorly-expressed SLC19A3.2 gene to provide enough thiamine transporters to compensate for the low disease-related expression of SLC19A3.1 [133].

Several disease states are also associated with thiamine deficiency. One such disease is congestive heart failure (CHF), which in humans has been found to be associated with a decreased thiamine status compared to healthy, age- and gender-matched controls [61]. Cardiac failure has been experimentally induced in dogs given a thiamine-deficient diet [135], though some dogs in this study did not develop heart failure despite ingesting a thiamine-deficient diet. The diet that was fed was not complete and balanced and was potentially deficient in other nutrients as well, which may have confounded results. The relationship between CHF and thiamine deficiency in dogs and cats therefore requires further study. Moreover, in patients with CHF, the use of certain medications, such as diuretics which are discussed below, should also be considered when examining thiamine status.

### 1.6.2.3 – Excessive Thiamine Excretion

Because of the kidneys' role in excretion and re-uptake of thiamine, diseases affecting the kidneys have an effect on thiamine status. In humans, decreased renal function associated with type 1 and type 2 diabetes has been linked to thiamine deficiency due to excess excretion of thiamine compared to healthy individuals [64]. Moreover, in any disease associated with polyuria, the rate of urinary excretion is expected to be increased due to thiamine's water-soluble nature. Also, in humans, a link exists between chronic kidney disease (CKD) and thiamine deficiency [78]. Clinically, CKD is associated with polyuria and polydipsia in dogs and cats, and can also result in anorexia or hyporexia [136], all of which are risk factors of thiamine deficiency. Despite this, a recent study in 19 dogs with CKD showed that these dogs had a higher blood TDP concentration compared to 47 healthy controls [137]. The authors hypothesized that decreased renal function led to lower urinary thiamine excretion, though they did not assess rates of thiamine excretion between dogs with CKD and controls. More research is therefore required to determine the effect of CKD on thiamine excretion. Research in humans has also found that dialysis may deplete B vitamin concentrations in patients with CKD, though the exact vitamins affected and the severity of depletion requires more study [138].

Additionally, diuretics may increase the risk for thiamine deficiency due to the increased urinary flow rates associated with the use of diuretics. A study examining urinary thiamine excretion in rats compared different diuretics at increasing dosages, and found that furosemide and mannitol, as well as volume loading with isotonic saline solution, led to significantly higher urinary thiamine excretion compared to baseline values [63]. Increasing thiamine excretion was found to be related to increasing urine output, and urinary thiamine losses also tended to increase with increasing diuretic dosages. The authors reported that

other diuretics tested in this study (chlorothiazide, acetazolamide, and amiloride) also showed higher urinary thiamine excretion compared to baseline, but detailed data for these medications were not provided and statistical significance was not reported. Furosemide has also been found to lead to poor thiamine uptake in cardiac cells of rats [139, 140], whereas high concentrations of amiloride are associated with poor in vitro thiamine absorption in the human small intestine [141].

### **1.6.3 – Clinical Signs of Thiamine Deficiency**

Signs of thiamine deficiency tend to be non-specific and can involve multiple organ systems [142], which can make the diagnosis challenging. In general, however, signs of thiamine deficiency in both dogs and cats are split into three progressive stages that are seen in both species: an induction stage, a critical stage, and a terminal stage [27].

The first stage, induction, occurs within one to two weeks of deficiency, and is characterized by a combination of vomiting, lethargy, and hyporexia or anorexia, though the animal's behaviour may remain otherwise unchanged [10, 65, 143-145]. Few other signs occur at this stage. However, weight loss due to chronic poor food intake may be noted [12, 65].

If deficiency is not reversed during the induction stage, the animal will enter the critical stage, and signs of nervous system damage will appear. In dogs and cats, these can include ataxia, paraparesis, nystagmus, delayed pupillary light response and blindness, recumbency, and increasingly poor proprioception, as well as seizures [10, 11, 15, 65, 69, 143, 146]. Cats may also exhibit cervical ventroflexion [143, 146] and

dyspnea [15, 143]. Cardiovascular signs during chronic deficiency may include arrhythmias and bradycardia [147].

The terminal stage of thiamine deficiency begins after approximately a month of severe deficiency, and involves a rapid worsening of signs until death occurs. It is fatal within a few days unless supplementation occurs immediately [142]. Most cases of thiamine deficiency are diagnosed at the terminal stage [142].

#### **1.6.4 – Diagnosis of Thiamine Deficiency**

Thiamine deficiency is poorly reflected on routine bloodwork. A complete blood count and serum biochemistry profile may be unremarkable in thiamine-deficient animals, even after prolonged deficiency [11, 13, 65, 146]. In humans, hyperlactatemia has been noted as a possible symptom of thiamine deficiency, which does not respond to treatment with sodium bicarbonate and can be aggravated by administration of glucose [58, 59]. Elevated lactate concentrations in urine may occur in dogs with thiamine deficiency [65, 147], but these are not always present in cats [66]. High concentrations of organic acids that indicate impairment in thiamine metabolism may be used to diagnose thiamine deficiency [65, 66]. As well, elevated lactate concentrations in plasma and CSF are not always present in dogs during thiamine deficiency [133], and CSF results can be unremarkable [13, 143, 146]. Blood pyruvate concentrations may be elevated in dogs with thiamine deficiency [147].

Analysis of thiamine status in veterinary patients is likely not regularly conducted, as thiamine status tends to not be reported in veterinary case reports pertaining to suspected thiamine deficiency. This may be due

to the lack of established reference range for thiamine status in dogs and cats, as well as poor availability of laboratories that routinely conduct thiamine analyses. Instead, thiamine deficiency in dogs and cats is typically diagnosed based on clinical signs and the presence of risk factors (e.g., unbalanced diet), and diagnosis is confirmed if signs reverse after thiamine supplementation [11, 14, 65, 66, 146, 148, 149]. An assessment of risk factors for thiamine deficiency should be accompanied by a nutritional assessment, such as the World Small Animal Veterinary Association (WSAVA) nutritional assessment guidelines, to rule out a nutritional cause [150]. Patients consuming raw or cooked homemade diets may be more likely to develop nutritional deficiencies due to consuming nutritionally unbalanced diets [82], which can include deficient thiamine intake. However, thiamine deficiency can also occur with commercial diets. Suspicion of a dietary cause of deficiency can be confirmed by analyzing a sample of the patient's food for its total thiamine concentration.

Magnetic resonance imaging (MRI) can be used to reveal brain lesions commonly seen in thiamine deficiency. These tend to consist of symmetrical, bilateral lesions in the brainstem that primarily affect grey matter [66, 151]. On histopathology, lesions are especially present in the nuclei of the caudal colliculi, and may either be spongy or involve cell degeneration and hypertrophy [10, 11, 15, 66, 151]. Lesions may also be present in the cerebellar noduli, rostral colliculi, and suprasplenic gyri of the caudal cortex, and the medial vestibular nuclei [10, 11, 145, 146, 151]. One report revealed unremarkable MRI findings despite presenting with clinical signs of thiamine deficiency in a cat, though it is unclear if signs reversed due to the cat having received supplemental thiamine for several days prior to MRI, or if no abnormalities had been present prior to supplementation [143].

### 1.6.5 – Treatment of Thiamine Deficiency

Treatment of thiamine deficiency requires thiamine supplementation, either as a vitamin supplement or included in a complete and balanced diet. If thiamine deficiency is suspected due to an unbalanced diet, and if the suspected deficiency is discovered before the onset of advanced clinical signs, administration of supplemental thiamine and a change to a complete and balanced diet may be all that is required to prevent the appearance of further signs of deficiency [10, 148].

As one of the signs of thiamine deficiency is anorexia [27], oral administration of thiamine as part of a complete and balanced commercial canned recovery diet or a veterinary liquid diet may require a feeding tube if the patient refuses or is unable to eat independently. Enteral feeding of patients with prolonged poor food intake should begin at one-third of the patient's resting energy requirements [ $\text{RER} = 70 \times (\text{body weight}_{\text{kg}}^{0.75})$ ], then increase to 2/3 and then to the patient's full RER every 24 hours or slower in order to avoid metabolic complications [152]. Veterinary liquid diets tend to contain adequate concentrations of thiamine. A recent study found thiamine in sufficient concentrations in all but one analyzed diet [153]. Adequate thiamine supplementation is therefore expected in the majority of veterinary liquid diets. This suggests that supplying thiamine through a complete and balanced veterinary liquid diet may be a viable option for patients that are able to be fed enterally. An oral thiamine supplement may also be administered to patients; however, large oral doses of synthetic thiamine may be poorly absorbed in dogs [154], which would suggest that other forms of supplementation may be more effective.

Parenteral thiamine, typically in the form of thiamine hydrochloride, may be administered via

intramuscular (IM), intravenous (IV), or subcutaneous (SQ) injections [142]. The ideal dose for supplemental thiamine is not currently known, and dosages reported in the literature for parenteral supplementation vary. In adult cats, dosages range from 25-300 mg per day [13, 14, 66, 143, 148], while in adult dogs they can range from 50-1250 mg per day [11, 65, 69]. Supplementation may begin with a loading dose of thiamine with subsequent smaller doses, or may utilize consistent dosages throughout treatment. However, dosages tend to be reported per animal rather than per kilogram, and the routes of thiamine administration vary between cases, therefore comparisons of precise dosages are difficult. There have been reports of severe reactions, such as anaphylaxis, neuromuscular or ganglionic blockade, apnea, hypotension, and death associated with IV administration in dogs and cats, and in humans [27, 30, 155], and it is therefore discouraged as a method of thiamine supplementation.

#### **1.6.6 – Prognosis and Outcome of Thiamine Deficiency**

The severity of thiamine deficiency may have an impact on outcomes. A retrospective study of human hospital patients found that mortality rates were higher in human patients with severe thiamine deficiency compared to patients with slight or no deficiency [156]. However, due to the retrospective nature of this study, the researchers were unable to quantify illness severity or nutritional status in patients.

Signs of thiamine deficiency may begin to resolve within several hours after supplementation [11, 66, 144, 148]. One case series found that 76% (13/17) of cats being fed a thiamine-deficient diet showed significant improvement within one week when treatment consisted of supplementation in addition to a diet change to a complete and balanced feline diet [143]. Resolution of MRI signs may be seen within days of beginning

thiamine supplementation [14, 65, 143], but MRI abnormalities may persist for several weeks [65].

Additionally, clinical signs may remain despite absence of MRI signs [65, 143]. Some neurological signs may persist for days to months after treatment ends [12, 27, 65, 66, 143], and neurological signs such as mild ataxia may persist for up to two years after treatment [143].

### **1.6.7 – Prevention of Thiamine Deficiency**

As thiamine is a dietary requirement in mammals, consistent daily intake is essential to prevent deficiency. This can be through a complete and balanced commercial diet, or a nutritionally balanced homemade diet formulated by a veterinary nutritionist to meet a dog or cat's nutrient requirements for their respective life stage. Signs of deficiency in dogs and cats are non-specific and involve multiple organ systems, thus thiamine deficiency is difficult to diagnose. Quick identification of clinical signs of thiamine deficiency are therefore critical in diagnosing and treating thiamine deficiency to ensure the survival of the deficient dog or cat.

The prevalence of thiamine deficiency in companion animals presenting to veterinary hospitals and ICUs is currently unknown. Parenteral thiamine is routinely administered in human hospitals for patients considered to be at high risk for deficiency [155], but the same is not necessarily true for dogs and cats presenting to veterinary hospitals. Thus, the need for routine thiamine administration in dogs and cats must be assessed in order to determine whether thiamine injections should be included as a routine part of medical care for dogs and cats presenting to veterinary ICUs.

## **1.7 – Objectives and Hypotheses**

### **1.7.1 – Study 1: Thiamine Retention in Extruded Dog and Cat Food**

Since pet owners may be tempted to purchase large bags of kibble and freeze the excess to save on costs, the purpose of this study was to determine the effect of long-term freezing on thiamine retention in extruded dry dog and cat food when compared to storage at room temperature. Because thiamine is sensitive to several types of processing and environmental conditions, it was hypothesized that freezing would lead to poorer thiamine retention, as subsequent thawing may lead to extra thiamine losses compared to room-temperature kibble that is not subject to temperature changes.

### **1.7.2 – Study 2: Thiamine Status in Dogs and Cats**

There have been few investigations of thiamine status in dogs and cats to date, and a reference range for thiamine status has yet to be developed in either species. Thus, the first objective of this study was to determine thiamine status for healthy adult dogs and cats in the Guelph, Ontario area. Additionally, as thiamine deficiency has an important role in the development or severity of several diseases in human medicine, the second objective of this study was to determine thiamine status in dogs and cats presenting to a veterinary intensive care unit, as well as the impact of supplementation on thiamine status in these animals. It was hypothesized that critically ill dogs and cats would have a poorer thiamine status compared to the above reference range, and that supplementation would increase thiamine status in these patients.

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## CHAPTER 2: EFFECT OF STORAGE TIME AND TEMPERATURE ON THIAMINE CONCENTRATIONS IN EXTRUDED COMMERCIAL CANINE AND FELINE DIETS: A PILOT STUDY

### 2.1 – Abstract

**Objective:** To examine rates of thiamine degradation in commercial pet foods after three months of storage at room temperature or frozen.

**Design:** Prospective study.

**Sample:** Three canine and three feline extruded dry diets.

**Procedures:** Samples of each diet were obtained and were assessed for thiamine concentration using fluorometry. Each sample was then split into two subsamples, one stored at room temperature (24°C) and the other frozen at -20°C. Each subsample was analyzed after five weeks, and after three months, for thiamine concentration. The effect of diet, storage temperature and storage time on thiamine concentration were assessed. A linear mixed model examined differences in thiamine concentrations in relation to differences in time, storage temperature, and storage time when data were pooled among all time points.

**Results:** Thiamine concentrations in each diet significantly differed from the other diets ( $p < 0.01$ ). There was no significant difference between frozen and room temperature samples ( $p = 0.46$ ), but samples contained significantly more thiamine at three months than at five weeks ( $p = 0.03$ ). There was no interaction between storage temperature and time ( $p = 0.92$ ).

**Conclusions and Clinical Relevance:** Diets were formulated to different specifications, and thus contained different thiamine concentrations. There was no appreciable difference in thiamine concentrations between room temperature and frozen samples, suggesting that freezing may have negligible effects on thiamine in extruded diets. The increased thiamine over time could not be definitively explained, though was likely related to uncertainty in the analysis method. As thiamine concentrations

remained within the Association of American Feed Control Officials recommendations at all times, freezing extruded dry food for three months may not lead to an increased rate of thiamine degradation in extruded pet food or risk of thiamine deficiency in pets.

## **2.2 – Introduction**

Thiamine (vitamin B<sub>1</sub>) is an essential dietary nutrient in dogs and cats [1]. It has a role in carbohydrate metabolism, nucleotide synthesis, and nervous function [2]. Dietary thiamine is present in plant sources in its unphosphorylated form (free thiamine), and in animal sources it is predominantly found as thiamine diphosphate (TDP). It can also be added to foods as a synthetic supplement [2].

Thiamine readily degrades during processing, as well as during exposure to heat, neutral and alkaline pH, and during oxidation [2-5]. Thiaminases, which are thiamine-destroying enzymes, are present in raw fish, and can also lead to thiamine degradation [2]. Thiamine degradation as a result of freezing has received less attention in the literature, and results are difficult to interpret as many studies examine the effects of both freezing and at least one other parameter (e.g., blanching, canning, cooking) on thiamine degradation [6-9]. According to recommendations set forth by the Association of American Feed Control Officials (AAFCO) for commercial dog and cat foods produced in North America, adult dogs and cats should receive at least 2.25 and 5.6 mg thiamine/kg of dry matter (DM) daily, respectively [10]. This has been increased from the AAFCO's 2014 recommendations of 1.0 and 5.0 mg thiamine/kg DM for adult dogs and cats, respectively [11]. No maximum recommendation has been determined for thiamine intake.

Thiamine deficiency seems to be of special concern in canned feline diets, as a recent study found that

13.3% of canned feline diets had thiamine concentrations below the AAFCO 2014 recommended minimum thiamine concentrations despite having an AAFCO adequacy statement [12]. Indeed, since 2010 there have been 6 pet food recalls due to thiamine deficiency in North America, which have involved a total of 17 canned feline diets, 5 extruded feline diets, and one raw feline diet [13].

Pet owners may freeze extruded dog and cat foods in order to preserve freshness and palatability when purchasing large volumes of food. It is typically cheaper to purchase larger volumes of food; however, small dogs and cats may not consume the large volume of food purchased while the food is still fresh and palatable. The potential for thiamine degradation in extruded canine and feline diets after freezing has not been well-examined and is therefore the focus of this study. The purpose of this study was to compare the degree of thiamine degradation in commercial extruded canine and feline diets after storage at freezing and at room temperatures.

### **2.3 – Methods**

*Diets* – Samples of three canine<sup>a-c</sup> and three feline<sup>d-f</sup> extruded diets were obtained from a single manufacturer (ON, Canada) within one month of being manufactured. All diets were formulated to meet AAFCO nutrient profiles for all life stages of dogs or cats, respectively. Feline diets consisted of fish-, chicken-, and poultry-based diets, and canine diets consisted of fish-, lamb-, and poultry-based diets. Fish diets contained salmon and trout as primary protein sources, and poultry diets contained chicken and turkey. The guaranteed analyses and baseline thiamine concentrations for all diets are provided in Table 2.1.

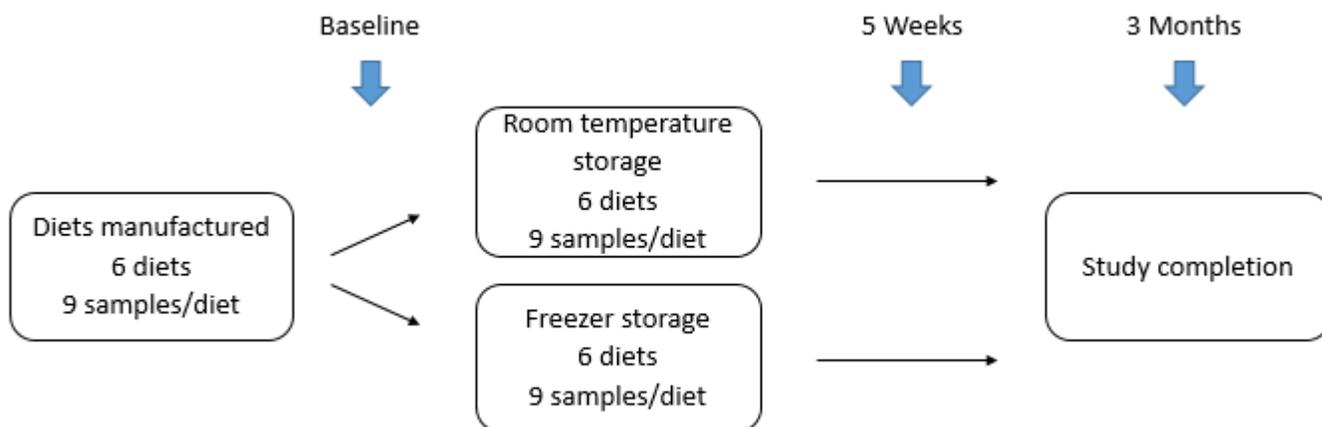
**Table 2.1.** Guaranteed analyses and baseline thiamine concentrations of three canine and three feline extruded diets.

Parameter	Canine Diets			Feline Diets		
	Poultry <sup>a</sup>	Fish <sup>b</sup>	Lamb <sup>c</sup>	Chicken <sup>d</sup>	Fish <sup>e</sup>	Poultry <sup>f</sup>
CP, % min	32.0	36.0	30.0	32.0	36.0	36.0
EE, % min	17.0	17.0	16.0	18.0	17.0	19.0
CF, % of total	4.0	2.9	4.4	3.0	3.2	3.2
Moisture, % max	10.0	10.0	10.0	10.0	10.0	10.0
Energy Density (kcal/kg ME)	3853	3993	3764	4032	3988	4082
Thiamine (mg/kg as fed)	9.4	12.0	10.6	13.4	18.3	14.7

CP – crude protein, EE – ether extract, CF – crude fibre, ME – metabolisable energy

*Sampling and storage* – Samples were obtained from the six diets by the manufacturer, within one month of manufacturing. During the production process, nine samples were obtained of each diet for a total of 54 samples. For each diet, three samples were collected at the start of each production run, three from the middle, and three from the end. An initial subsample was taken from each of these nine samples and the subsamples were pooled per diet, homogenized and analyzed for thiamine concentration. Next, the 54 samples were split, with one half of each sample placed in a -20°C freezer and the other half stored at room temperature (24°C). All samples were stored in airtight bags, and were double-bagged. Bags were stored in opaque containers to prevent light exposure. At 5 weeks, and again at 3 months, a subsample was taken from each of the frozen and room temperature samples. Again subsamples were pooled per diet and storage temperature and each subsample was homogenized prior to thiamine concentration determination.

Sampling methodology is described in Figure 2.1.



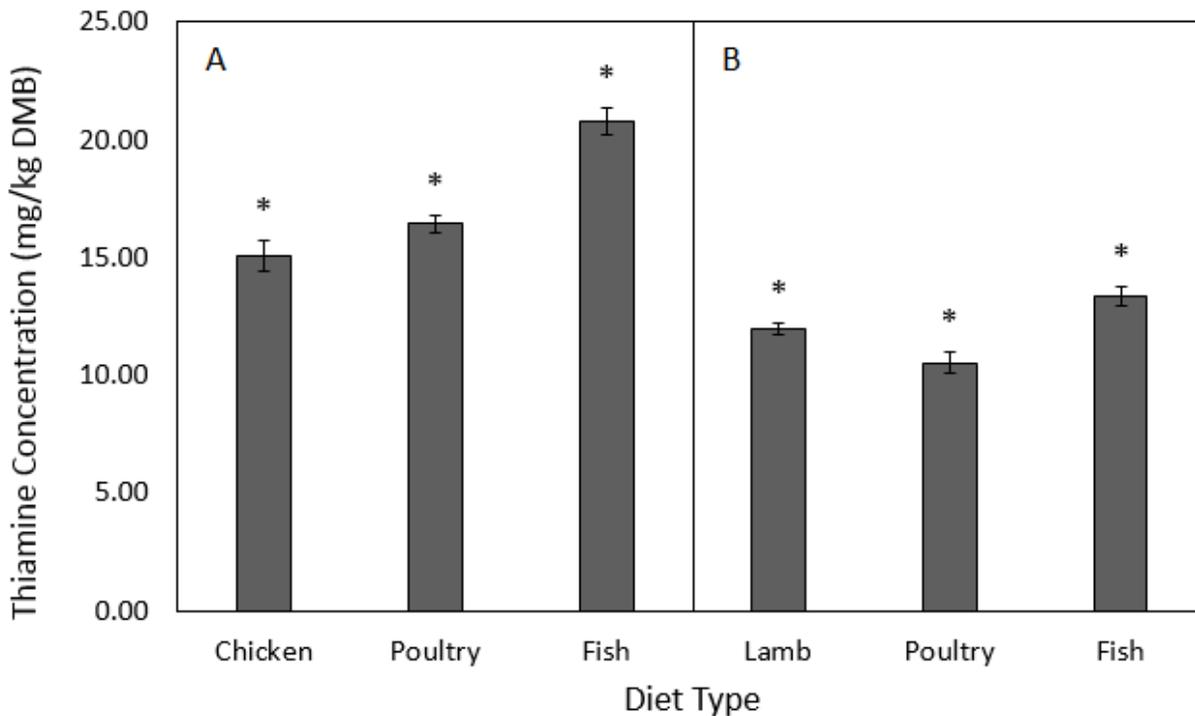
**Figure 2.1.** Sampling protocol for thiamine analysis of three canine and three feline extruded diets. Sampling time points are shown with arrows. Thiamine concentrations of each diet were analyzed upon study commencement (Baseline), after which samples were split in half into room temperature and freezer treatment. Thiamine was analyzed again after five weeks and three months. Thiamine concentrations of each diet represent nine pooled subsamples.

*Analytical methods* – All analyses for thiamine concentration were conducted at a commercial laboratory<sup>g</sup> via fluorometry<sup>h</sup>. The method of analysis had a relative standard deviation of 4.4%. The crude fibre (CF) content of all diets was analyzed at a commercial laboratory<sup>i</sup> using a filter bag technique<sup>j</sup>. The CF, on a dry matter basis (DMB), was used to determine the energy density of each diet using the respective National Research Council (NRC) equation for canine or feline metabolisable energy (ME) [14].

*Statistical analysis* – Analyses were conducted using commercial statistical software<sup>k</sup>. Data were assessed for normality using Shapiro-Wilk tests and the residuals of the model. All data were normally distributed; thus, parametric analyses were conducted. Data were assessed using a linear mixed model to analyze differences in thiamine concentration in relation to the specific diets as well as storage time (5 weeks vs. 3 months) and storage temperature (room temperature vs. freezer). A priori specific comparisons of interest were conducted to assess differences between diets.

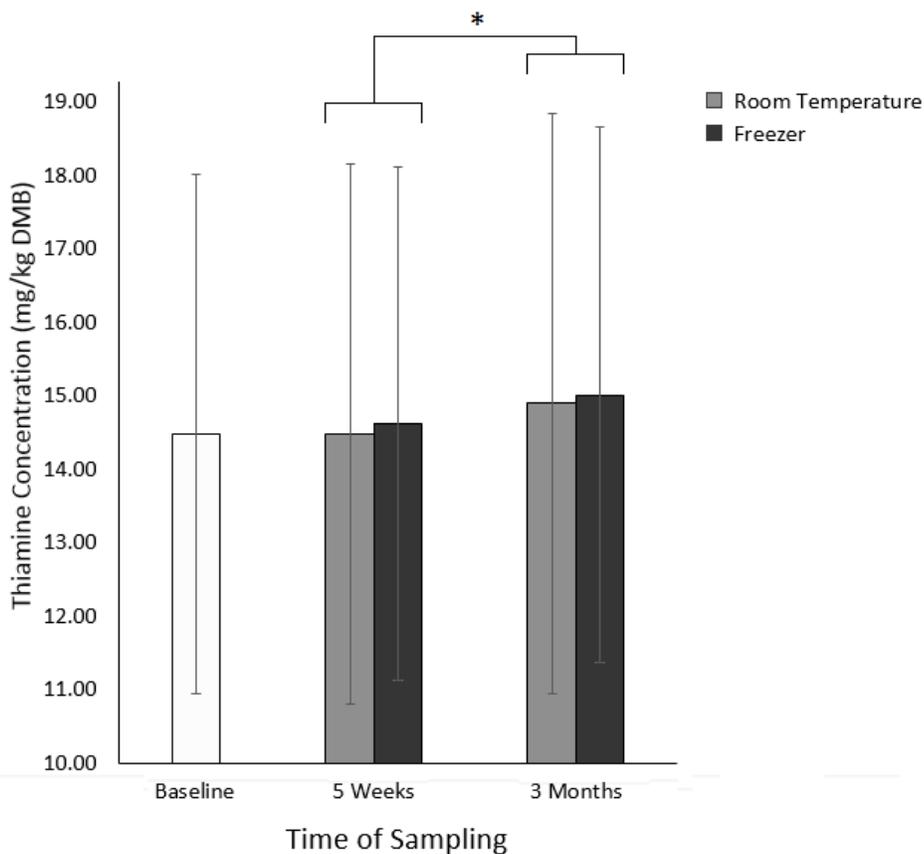
## 2.4 – Results

Diets were manufactured prior to AAFCO’s 2016 increase in dietary thiamine recommendations; however, thiamine concentrations in each canine and feline diet during the entire study period remained above the thiamine concentrations recommended by AAFCO in 2017 for dogs and cats, respectively. Each diet’s average thiamine concentration, calculated from thiamine concentrations at three time points and two storage temperatures ( $n = 5$  for each diet), was significantly different from each of the other diets ( $p < 0.01$  for each comparison) (Figure 2.2). The three feline diets (range: 15.1 – 20.8 mg/kg DMB) contained more thiamine than the three canine diets (range: 10.6 – 13.4 mg/kg DMB).



**Figure 2.2.** Thiamine concentrations (mg/kg DMB) of samples of A) three feline extruded diets; and B) three canine extruded diets formulated with different protein sources. Each bar represents average thiamine concentration  $\pm$  standard deviation of three time points and two storage temperatures ( $n=5$ ) for each separate diet. An asterisk (\*) denotes significantly different thiamine concentrations between diets compared to all other diets ( $p < 0.01$ ).

Comparing the six diets at each temperature (room temperature, frozen) and each time point (5 weeks, 3 months) showed no overall significant difference ( $p = 0.13$ ). Also, interactions between temperature and time were not observed ( $p = 0.92$ ). However, a significant increase in thiamine concentration was present between 5 weeks and 3 months when thiamine concentrations for all diets and temperatures were averaged per time point ( $p = 0.03$ ,  $n = 12$ ). There was no difference in thiamine concentration between temperatures when thiamine concentrations for all diets at each time point were averaged per temperature ( $p = 0.46$ ,  $n = 12$ ). This relationship is presented in Figure 2.3.



**Figure 2.3.** Thiamine concentrations (mg/kg DMB) of frozen and room temperature extruded dry food samples upon study commencement, and after 5 weeks and 3 months for each storage temperature. Each bar represents an average thiamine concentrations  $\pm$  standard deviation from all diets ( $n = 6$ ) at each time point and for each temperature storage. Thiamine concentrations were significantly different between the 5-week and 3-month sampling time. There was no significant effect of storage temperature at any time-point or between any two time-points. Significance at  $p < 0.05$  is marked with an asterisk (\*).

## 2.5 – Discussion

Each of the six extruded diets evaluated in the present study were formulated using different ingredients to meet species-specific AAFCO recommendations for all life stages [10]. Therefore it was anticipated that each diet would contain significantly different thiamine concentrations compared to the other diets, but would also still be in accordance with AAFCO recommendations. Additionally, as cats have higher thiamine requirements than dogs [1], higher thiamine concentrations in feline diets compared to canine diets were also expected.

The fish-based diets evaluated in the present study included raw fish as ingredients (deboned trout and deboned salmon). Thiaminases present in the flesh of raw fish can reduce thiamine concentrations of ingredients prior to denaturing of thiaminases by the high heat that occurs with extrusion [2]. However, both the canine and feline fish-based diets contained thiamine concentrations above AAFCO recommendations. This could be due to additional supplementation of thiamine during processing to compensate for losses from utilizing raw fish ingredients, but more research is required to determine the relationship between protein source and resultant thiamine concentration in extruded dog and cat food.

A summary of previous research examining thiamine retention in extruded dry dog and cat foods reports that retention after 18 months of storage is 34.2% and 57.5%, respectively [15]. This suggests that there is a steady decrease in thiamine concentration over time, although this summary did not describe storage conditions for the diets. In the present study, this expected decrease in thiamine concentrations was not seen over time, but changes in thiamine concentrations were only quantified over a three-month period and potential effects of longer storage could not be assessed. In contrast, thiamine concentrations were

significantly higher after three months than after five weeks. A previous study in frozen spinach found that thiamine concentrations increased after freezing for the entirety of a 40-day study period [16], though the authors were unable to determine the reason for the initial increase. However, thiamine fluctuations in frozen vegetables may not be directly comparable to extruded pet foods.

In the present study, both the frozen and room temperature samples demonstrated a slight, though statistically significant, increase in thiamine concentration over time. It is therefore unlikely that increased thiamine concentrations were due to freezing. Additionally, small losses in moisture content over time can occur in foods as a response to air temperature and humidity [17] or as a consequence of freezing [18], which could have led to a slight increase in thiamine concentrations. Moisture change may therefore have influenced thiamine concentrations over the course of the study period. However, extruded pet food has a low moisture content between 3-11% [15], and samples in the present study were stored in sealed containers to minimize exposure to air. The difference between thiamine concentrations at the five-week and three-month time point may also be a function of the inherent variation in the analysis methodology, as results of thiamine analysis in pet foods have historically been found to fluctuate between laboratories and between replicates of the same sample [19].

As a pilot study, there were limitations to the study design that would need to be addressed in a broader analysis of thiamine concentrations in dog and cat foods. First, small sample sizes and a lack of replicates decreased the power of the statistical analysis, and future research should utilize larger sample sizes and analyze thiamine concentrations in duplicate or triplicate. Additionally, moisture content could not be accounted for in this study and future research should measure potential changes in moisture when assessing thiamine concentrations of canine and feline diets. Furthermore, as thiamine degrades when

oxidized and when exposed to light and heat [2], storage media could affect thiamine retention in extruded dry pet foods. Pet foods are sold in a variety of packaging types, which suggests that utilization of different storage containers and materials could lead to variable rates of thiamine degradation in these foods. The effect of storage containers on thiamine concentrations in extruded dry dog and cat food was not assessed in this study, and is an area requiring further investigation.

Pet owners may buy large bags of extruded dry food and freeze them to preserve freshness and palatability. While thiamine in foods generally degrades during storage, the results of this pilot study suggest that there may be minimal clinical relevance of freezing extruded dry pet food compared to storage at room temperature for at least three months. Thiamine concentrations of all canine and feline extruded diets remained above their respective AAFCO recommendations, suggesting that dogs and cats receiving these diets will receive adequate thiamine regardless of the storage method that is used. However, more research is needed to determine the effect of temperature, storage containers, and longer storage periods on thiamine concentrations in commercial pet foods.

## **2.6 – Acknowledgements**

The authors would like to thank William Sears for assistance with statistical analysis, and Brittany Martin for assistance with sample preparation.

## 2.7 – Footnotes

- a. Canine poultry diet ingredients – deboned turkey, chicken meal, lentils, green peas, chickpeas, whole eggs, chicken fat, natural chicken flavour, deboned duck, flaxseed, pumpkin, broccoli, choline chloride, salt, pomegranate, raspberries, kale, chicory root extract, dried kelp, vitamins and minerals (vitamin E, vitamin A, vitamin D<sub>3</sub>, vitamin B<sub>3</sub>, vitamin C, vitamin B<sub>5</sub>, vitamin B<sub>1</sub>, vitamin B<sub>2</sub>, beta-carotene, vitamin B<sub>6</sub>, vitamin B<sub>9</sub>, vitamin B<sub>7</sub>, vitamin B<sub>12</sub>, zinc proteinate, ferrous sulfate, iron proteinate, zinc oxide, copper proteinate, copper sulfate, manganese proteinate, manganous oxide, calcium iodate, sodium selenite), *Yucca schidigera*, green lipped mussel, dried rosemary
- b. Canine fish diet ingredients – deboned trout, salmon meal, green peas, menhaden fish meal, chickpeas, lentils, canola oil, deboned salmon, natural flavour, salmon oil, apples, carrots, choline chloride, butternut squash, cranberries, blueberries, blackberries, kale, dried kelp, chicory root extract, vitamins and minerals (vitamin E, vitamin A, vitamin D<sub>3</sub>, vitamin B<sub>3</sub>, vitamin C, vitamin B<sub>5</sub>, vitamin B<sub>1</sub>, vitamin B<sub>2</sub>, beta-carotene, vitamin B<sub>6</sub>, vitamin B<sub>9</sub>, vitamin B<sub>7</sub>, vitamin B<sub>12</sub>, zinc proteinate, ferrous sulfate, iron proteinate, zinc oxide, copper proteinate, copper sulfate, manganese proteinate, manganous oxide, calcium iodate, sodium selenite), *Yucca schidigera*, green lipped mussel, dried rosemary
- c. Canine lamb diet ingredients – deboned lamb, lamb meal, lentils, green peas, chickpeas, pea protein, canola oil, natural flavour, apples, flaxseed, butternut squash, pumpkin, broccoli, DL methionine, choline chloride, salt, chicory root extract, dried kelp, vitamins and minerals (vitamin E, vitamin A, vitamin D<sub>3</sub>, vitamin B<sub>3</sub>, vitamin C, vitamin B<sub>5</sub>, vitamin B<sub>1</sub>, vitamin B<sub>2</sub>, beta-carotene, vitamin B<sub>6</sub>, vitamin B<sub>9</sub>, vitamin B<sub>7</sub>, vitamin B<sub>12</sub>, zinc proteinate, ferrous sulfate, iron proteinate, zinc oxide, copper proteinate, copper sulfate, manganese proteinate, manganous oxide, calcium iodate, sodium selenite), dried rosemary
- d. Feline chicken diet ingredients – chicken meal, chicken, brown rice, oatmeal, dehulled barley, chicken fat, salmon meal, whole egg, beet pulp, natural flavour, salmon oil, potatoes, tomatoes, hydrolyzed fish protein, brewer's yeast, salt, potassium chloride, calcium sulphate, phosphoric acid, chicory root extract, vitamins and minerals (vitamin E, vitamin C, vitamin B<sub>3</sub>, vitamin A, vitamin B<sub>1</sub>, vitamin B<sub>5</sub>, vitamin B<sub>6</sub>, vitamin B<sub>2</sub>, beta-carotene, vitamin D<sub>3</sub>, vitamin B<sub>9</sub>, vitamin B<sub>7</sub>, vitamin B<sub>12</sub>, zinc proteinate, ferrous sulfate, zinc oxide, iron proteinate, copper sulfate, copper proteinate, manganese proteinate, manganous oxide, calcium iodate, sodium selenite), glucosamine hydrochloride, taurine, *Yucca schidigera*, marigold extract (source of lutein), l-carnitine, dried rosemary
- e. Feline fish diet ingredients – deboned trout, salmon meal, menhaden fish meal, green peas, lentils, chickpeas, canola oil, deboned salmon, natural flavour, carrots, apples, butternut squash, choline chloride, cranberries, blueberries, blackberries, kale, chicory root extract, vitamins and minerals (vitamin E, vitamin C, vitamin B<sub>3</sub>, vitamin A, vitamin B<sub>1</sub>, vitamin B<sub>5</sub>, vitamin B<sub>6</sub>, vitamin B<sub>2</sub>, beta-carotene, vitamin D<sub>3</sub>, vitamin B<sub>9</sub>, vitamin B<sub>7</sub>, vitamin B<sub>12</sub>, zinc oxide, iron proteinate, copper sulfate, copper proteinate, manganese proteinate, manganous oxide, calcium iodate, sodium selenite), taurine, *Yucca schidigera*, dried rosemary

- f. Feline poultry diet ingredients – deboned turkey, chicken meal, lentils, whole eggs, green peas, chickpeas, chicken fat, natural chicken flavour, deboned duck, flaxseed, pumpkin, broccoli, choline chloride, cranberry meal, pomegranate, raspberries, kale, salt, chicory root extract, vitamins and minerals (vitamin E, vitamin C, vitamin B<sub>3</sub>, vitamin A, vitamin B<sub>1</sub>, vitamin B<sub>5</sub>, vitamin B<sub>6</sub>, vitamin B<sub>2</sub>, beta-carotene, vitamin D<sub>3</sub>, vitamin B<sub>9</sub>, vitamin B<sub>7</sub>, vitamin B<sub>12</sub>, zinc proteinate, ferrous sulfate, zinc oxide, iron proteinate, copper sulfate, copper proteinate, manganese proteinate, manganous oxide, calcium iodate, sodium selenite), DL methionine, taurine, *Yucca schidigera*, dried rosemary
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## CHAPTER 3: THIAMINE INTAKE AND STATUS IN A POPULATION OF HEALTHY DOGS AND CATS, AND COMPARISON WITH DOGS PRESENTING TO A VETERINARY REFERRAL HOSPITAL FOR INAPPETENCE

### 3.1 – Abstract

**Objective:** To determine thiamine status (thiamine diphosphate (TDP) and free thiamine) in a population of healthy dogs and cats and to determine thiamine status of inappetent dogs before and after supplementation.

**Design:** Clinical trial.

**Animals:** 22 healthy dogs and 8 healthy cats from the Guelph, Ontario area, as well as 11 dogs presenting to a tertiary referral hospital with a history of at least 3 days of inappetence.

**Procedures:** Healthy dogs and cats were fed extruded dry food with known thiamine concentrations. Food intake was recorded during the study period. After 21 days, blood thiamine status was analyzed. Blood thiamine status was also assessed in hospitalized dogs within 3 days of inappetence and following a minimum of four doses of thiamine administration.

**Results:** Average TDP status in healthy dogs and cats was  $732.1 \pm 109.9$  and  $2041.3 \pm 808.9$  nmol/L, respectively. Average free thiamine status was  $1320.1 \pm 189.6$  nmol/L in dogs; however, laboratory error prevented evaluation in cats. There was a significant decrease in TDP ( $p = 0.01$ ) and free thiamine ( $p = 0.02$ ) status with increasing age in healthy dogs. Free thiamine and TDP status were significantly related ( $p < 0.001$ ) in healthy dogs. Inappetent dogs had significantly higher TDP status ( $p = 0.001$ ) but not free thiamine status ( $p = 0.06$ ) compared to healthy dogs. Supplemented TDP status was significantly higher than baseline TDP status in inappetent dogs ( $p = 0.04$ ).

**Conclusions and Clinical Relevance:** More research is needed to determine a reference range for TDP and free thiamine in adult and senior dogs and cats. In hospitalized dogs, a short period of inappetence may not be sufficient to develop thiamine deficiency, though thiamine supplementation is a viable method to increase thiamine status in canine patients. Further research is needed to elucidate relationships between thiamine status and factors such as age, disease, and medication use in dogs and cats.

### 3.2 – Introduction

Thiamine (vitamin B<sub>1</sub>) is an essential dietary nutrient for dogs and cats as it cannot be synthesized in the body [1]. Tissue storage of thiamine is minimal and excess thiamine gets excreted through the urine [2], so thiamine must be ingested consistently to prevent deficiency.

Thiamine occurs in several forms. Unphosphorylated thiamine (free thiamine) and thiamine triphosphate (TTP) are thought to have a function in the nervous system [3]. Due to its ubiquitous nature in the body, TTP may have additional functions that are currently unknown [1, 4]. Thiamine monophosphate (TMP) is thought to be a storage form of thiamine that can be transformed to provide active forms [3]. Thiamine diphosphate (TDP), an active form of thiamine, acts as a cofactor in the Krebs's cycle and the pentose phosphate pathway, making it critical for energy metabolism and nucleotide production [1].

In North America, recommendations for dietary nutrient concentrations in dog and cat food are provided by the Association of American Feed Control Officials (AAFCO), which are based off of nutrient requirements provided by the National Research Council (NRC) [5]. As of 2016, AAFCO updated its thiamine recommendations for adult dog and cat diets to match the NRC's recommended allowance (RA)

of at least 2.25 and 5.6 mg thiamine per kg on a dry matter basis (DMB), or 0.56 and 1.40 mg/1000 kcal of metabolisable energy (ME) on a calorie basis, respectively for dogs and cats [6, 7]. There is currently no recommended safe upper limit for dietary thiamine intake. Thiamine toxicity due to over-supplementation of dietary thiamine has not been documented in dogs or cats in the veterinary literature.

To date, little research has examined thiamine status in companion animals. Blood thiamine status in humans is usually assessed using either erythrocyte transketolase activity (ETKA) or high-performance liquid chromatography (HPLC), though HPLC is considered the preferred method [8]. Currently, in veterinary practice, thiamine deficiency is not diagnosed using blood thiamine status. Instead, diagnosis is suspected based on clinical signs and the presence of risk factors upon nutritional assessment, and is confirmed by response to thiamine supplementation [9-12].

Initial signs of deficiency in dogs and cats include lethargy, decreased appetite, and vomiting, which can progress to neurologic signs such as ataxia, paraparesis, and seizures, and ultimately lead to death [9, 13-16]. Presently, there is no accepted supplementation protocol for thiamine, and a variety of dosages are recorded in the literature. In dogs, doses can range from 50-1250 mg/day [9, 12, 17]. In cats, they range from 25-300 mg/day [10, 11, 18-20]. It is difficult to compare dosages between case reports, as dosages are often provided per animal rather than per kilogram, and routes of thiamine administration can be oral, subcutaneous, intramuscular, or intravenous, or a combination of methods. Intravenous administration, however, is not recommended due to risk of severe reactions such as hypotension, apnea, and neuromuscular or ganglionic blockade [21-23]. Oral administration may not be as effective for acute treatment as other methods, as large doses of oral thiamine supplement may be poorly absorbed by dogs [24].

The first objective of the present study was to determine thiamine status (free thiamine and TDP) in healthy dogs and cats. The second objective of this study was to compare the thiamine status of healthy dogs with that of inappetent dogs, and to determine the effect of supplementation on thiamine status of hospitalized animals.

### **3.3 – Methods**

The experimental protocol of this study was approved by the University of Guelph's Animal Care and Use Committee (AUP#3184), and was in accordance with institutional and national guidelines for care and use of animals.

#### Experiment 1: Healthy Dogs and Cats

*Animals* – Client-owned dogs and cats from the Guelph, Ontario, region were recruited for this study between September 2015 and June 2016. Participants were at least one year old, had a body condition score (BCS) between 4-7/9 [25, 26], and had mild or no muscle wasting according to the muscle condition scoring (MCS) system recommended by the World Small Animal Veterinary Association Nutritional Assessment Guidelines [27, 28]. Animals were excluded if they had any medical conditions, were receiving medications (other than routine flea, tick, heartworm, and/or intestinal parasite prevention), if they had been hospitalized in the past year, or if they had food allergies precluding feeding an over-the-counter adult maintenance diet.

*Diets and feeding* – Three canine and three feline diets, each with different protein sources, were utilized for this study. Canine diets had lamb, poultry (chicken and turkey), and fish (salmon and trout) as predominant protein sources, while protein sources in feline diets consisted of chicken, poultry (chicken and turkey), and fish (salmon and trout). Diets are as previously described in Chapter 2 (Table 2.1). Samples of each diet were weighed to determine the average quantity of grams fitting into one standard measuring cup<sup>a</sup> in order to calculate the approximate kcal/cup based on the diets' energy density in kcal/kg. Diets were randomized prior to study commencement using a random number generator. Dogs and cats were assigned one of the canine or feline diets, respectively, based on order of enrollment. Owners were blinded to the diet chosen for their pets. Participants were weighed and owners were asked to describe their pets' activity levels, medical history, and diet history prior to enrollment. Based on weight and activity level, an appropriate NRC equation for caloric intake was selected for each participant [7]. The resultant daily caloric requirement was then divided by the kcal/cup for the assigned diet, and was rounded to whichever quarter or third of a cup was closest to the true value. Owners were instructed to transition their pet to their assigned study diet over a period of seven days, using a transition schedule where participants were fed 25% of the study diet and 75% of their current diet for two days, then 50% of each diet for two days, then 75% of the study diet and 25% of their current diet for two days before being put completely on the study diet. Participants were then fed solely the study diet for 14 days. Owners were instructed to refrain from providing treats or any other food, and were provided with a food log to record the amount of study diet eaten per day throughout the study period.

*Sample collection* –After at least 21 days (7 days transition + at least 14 days solely on the study diet), participants were fasted for 12h, after which BW, BCS, and MCS were re-assessed, and blood and urine were collected. Serum separator tubes were utilized to collect blood for serum biochemistry profiles and

EDTA-coated tubes were utilized for assessment of complete blood count (CBC), as well as for collection of blood for thiamine profile analysis. A routine urinalysis was conducted for each participant. Dogs and cats with abnormalities on serum biochemistry, CBC, or urinalysis were excluded from further analysis. Daily thiamine ingestion was determined by averaging thiamine intake over the 14-day study period.

### Experiment 2: Dogs and Cats with Reduced Appetite

*Animals* – Client-owned dogs and cats presenting to the Intensive Care Unit (ICU) at the Ontario Veterinary College’s Health Sciences Centre (Guelph, ON) between April 2015 and August 2016 were considered candidates for enrollment if they had a history of anorexia or hyporexia (<25% resting energy requirement) for at least three days, if they were at least one year old, had a hematocrit of at least 20%, had no recent history of red blood cell (RBC) or whole blood transfusions, and had no medical conditions precluding venipuncture or subcutaneous (SQ) thiamine supplementation. Reduced appetite was assessed through information provided by owners upon admission and by documents submitted by referring veterinarians, and was confirmed by further communication with owners.

*Sample collection and thiamine supplementation* – Patients were enrolled within 24-72h of hospitalization. Upon participant enrollment, each patient’s BCS and MCS were assessed. Signalment, differential diagnoses, and BW were recorded. At this time, EDTA blood was collected and refrigerated to provide a baseline thiamine status for inappetent animals. Thiamine supplementation was subsequently administered to a subset of patients that were suspected to require hospitalization for two or more days from the study commencement date. The supplement consisted of thiamine hydrochloride<sup>b</sup>. Cats received 100mg SQ thiamine q12h, and dogs received 50mg/10kg BW SQ q12h. Doses were diluted to 1mL with 0.9% NaCl prior to administration to prevent injection-related discomfort. Supplementation continued for four to five

doses, depending on length of hospital stay. At least 8h after the final dose, a second EDTA blood sample was collected and refrigerated for subsequent thiamine determination.

#### Determination of Thiamine Status

Blood samples collected for thiamine profile testing were immediately refrigerated and processed within 12h of collection. Prior to centrifugation, the centrifuge was cooled to 4°C. Blood was centrifuged at 1200rpm for 10 minutes to separate erythrocytes from the plasma and buffy coat. Following separation, the RBCs were washed with 12mL of saline and centrifuged at 1200rpm for 10 minutes. Excess saline was removed. This process was repeated three times. The hematocrit of the remaining RBCs was then determined. Samples were volume-adjusted with saline, if needed, in order to have a hematocrit between 48-52%. This was done either by adding saline to lower hematocrit, or by centrifuging again at 1200rpm for 10 minutes to remove saline to increase hematocrit. A 1mL aliquot of each volume-adjusted sample was placed in a cryovial and stored at -80°C until shipment.

Samples were shipped on dry ice to AniLytics Inc (Gaithersberg, MD) for analysis of free thiamine and TDP using high-performance liquid chromatography (HPLC). Briefly: samples were first mixed with an aqueous solution of 40% trichloroacetic acid (TCA), then vortexed and incubated, in the dark, at room temperature for 1h before centrifugation at 3000rpm for 15-20min to remove the clear supernate. The supernate was centrifuged at 10,000rpm for approximately 10min, after which 100-200µL of each supernate was removed, and 0.57mL of water saturated ether was added for each 100µL of extract. Samples were then vortexed and centrifuged at approximately 1500rpm for approximately 5min. Samples were stored at -50°C or below until the aqueous layer froze. The liquid ether layer was removed and samples were allowed to thaw. For each 100µL of sample, 5µL of potassium hexacyanoferrate was added,

then each sample was mixed and allowed to sit for approximately 5min, after which 5 $\mu$ L of 0.8M hydroxide was added. The sample was mixed again, then HPLC proceeded according to published methodology [29]. The method of analysis had an error of  $\pm$  15%. Results were reported as nmol/L.

### Statistical Analysis

Data were analyzed using commercial open-source software<sup>c</sup>. Normality was assessed graphically, using residuals of linear models, and using Shapiro-Wilk tests. Separate linear models were conducted to examine the effect of diet type, age, sex, and dietary thiamine intake on TDP and free thiamine status of healthy dogs and cats. Comparisons between thiamine status (TDP and free thiamine) of inappetent dogs with healthy dogs were conducted using independent samples t-tests or Mann-Whitney U tests.

Comparisons between thiamine status (TDP and free thiamine) during inappetence and after supplementation were assessed via paired t-tests or Wilcoxon sign rank tests. Data that were normally distributed were assessed with parametric analyses, and data that were not normally distributed were assessed using equivalent nonparametric analyses. Significance was defined as  $p < 0.05$ . Descriptive statistics are presented as mean  $\pm$  standard deviation for normally distributed data and as median (range) for non-normally distributed data.

## **3.4 – Results**

### Experiment 1 – Healthy Dogs and Cats

Twenty-two healthy dogs were enrolled in this study. Eight dogs were mixed breeds, three were golden retrievers, and the following breeds each had one representative: Weimaraner, Pug, Border Collie, Miniature Dachshund, Shetland Sheepdog, Miniature Poodle, Newfoundland Dog, Bernese Mountain Dog,

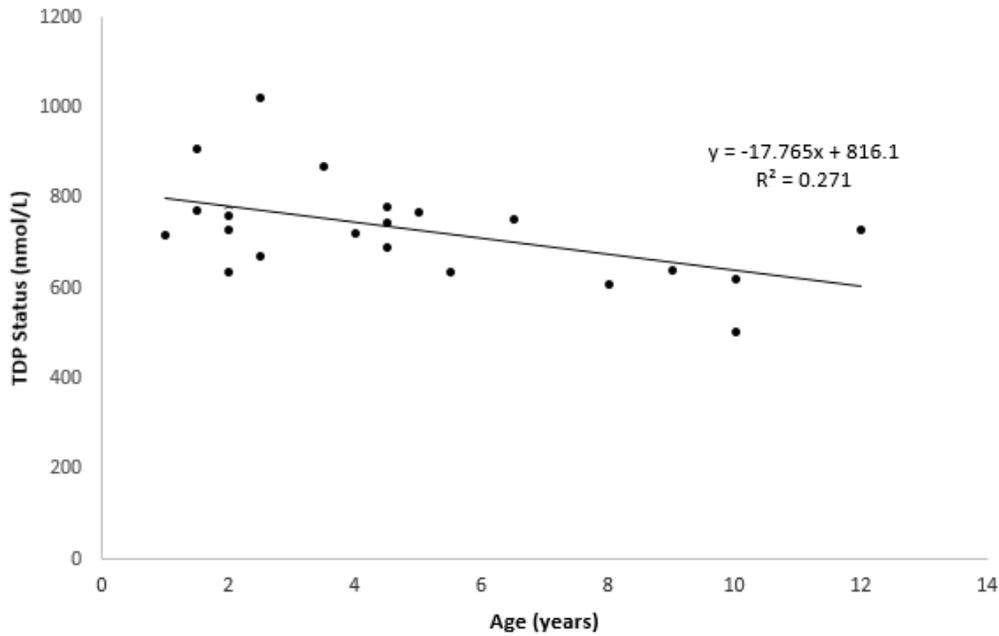
German Shepherd, Australian Shepherd, and Lakeland Terrier. Nine dogs were spayed females, and 13 dogs were males (12 neutered, 1 intact). The average age of participants was  $4.7 \pm 3.2$  years. The average BCS and MCS upon study completion were  $5.2 \pm 0.7$  and  $2.8 \pm 0.4$ , respectively.

Dogs were assigned the following diets: six lamb diet, nine fish diet, and seven poultry diet. Average daily thiamine intake was  $3.4 \pm 1.5$  mg/day as fed. Healthy dogs had a TDP and free thiamine status of  $732.1 \pm 109.9$  and  $1320.1 \pm 189.6$  nmol/L, respectively (Table 3.1). Thiamine diphosphate status was not affected by diet ( $p = 0.39$ ), sex ( $p = 0.84$ ), or thiamine intake ( $p = 0.74$ ). Free thiamine status was not affected by diet ( $p = 0.25$ ), sex ( $p = 0.77$ ), or thiamine intake ( $p = 0.76$ ). Thiamine diphosphate status declined with age ( $p = 0.01$ ; Figure 3.1), as did free thiamine status ( $p = 0.02$ ; Figure 3.2). There was a positive relationship between TDP status and free thiamine status ( $p < 0.001$ , Figure 3.3).

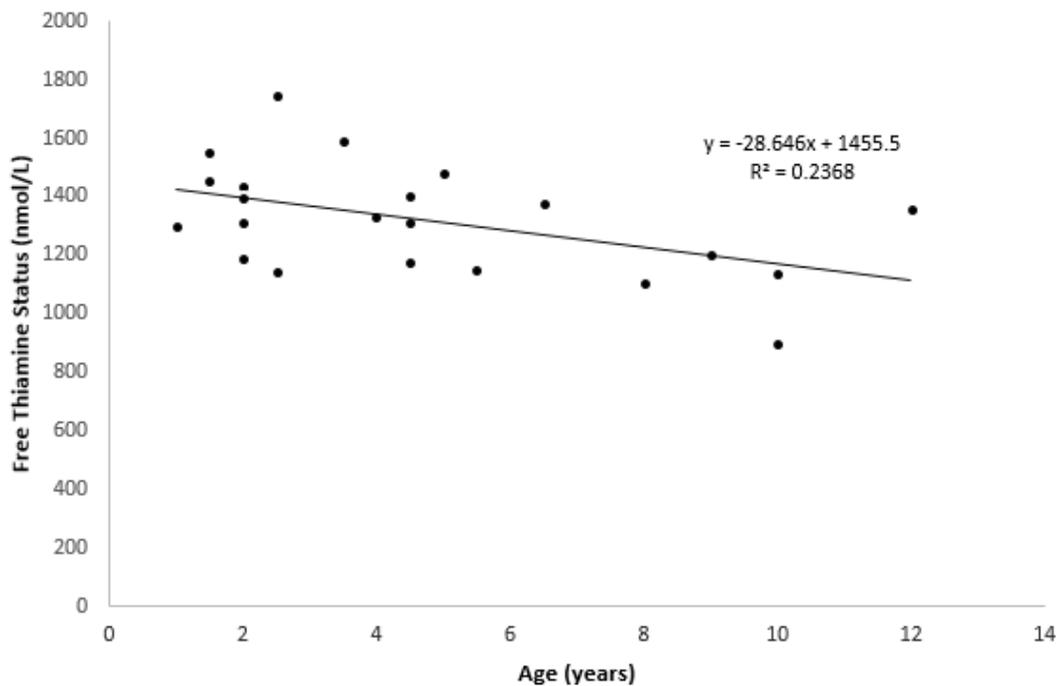
**Table 3.1.** Free thiamine and TDP status of healthy dogs and cats ingesting diets with known concentrations of thiamine. Free thiamine status could not be determined for cats. Thiamine status is presented as mean  $\pm$  s.d.

Parameter	Sample size	Free Thiamine Status (nmol/L)	TDP Status (nmol/L)
Dogs	22	$1320.1 \pm 189.6$	$732.1 \pm 109.9$
Diet			
Lamb	6	$1228.4 \pm 288.4$	$696.4 \pm 175.9$
Fish	9	$1394.6 \pm 143.5$	$771.1 \pm 82.7$
Poultry	7	$1303.0 \pm 109.5$	$712.6 \pm 58.4$
Sex			
Female	9	$1335.1 \pm 241.2$	$738.0 \pm 138.5$
Male	13	$1309.7 \pm 154.4$	$728.0 \pm 91.2$
Cats	8	NA	$2041.3 \pm 808.9$
Diet			
Chicken	1	NA	1715.8
Fish	3	NA	$2893.3 \pm 669.3$
Poultry	4	NA	$1483.8 \pm 229.2$
Sex			
Female	4	NA	$2352.6 \pm 1074.4$
Male	4	NA	$1730.1 \pm 337.8$

NA – result not available, TDP – thiamine diphosphate

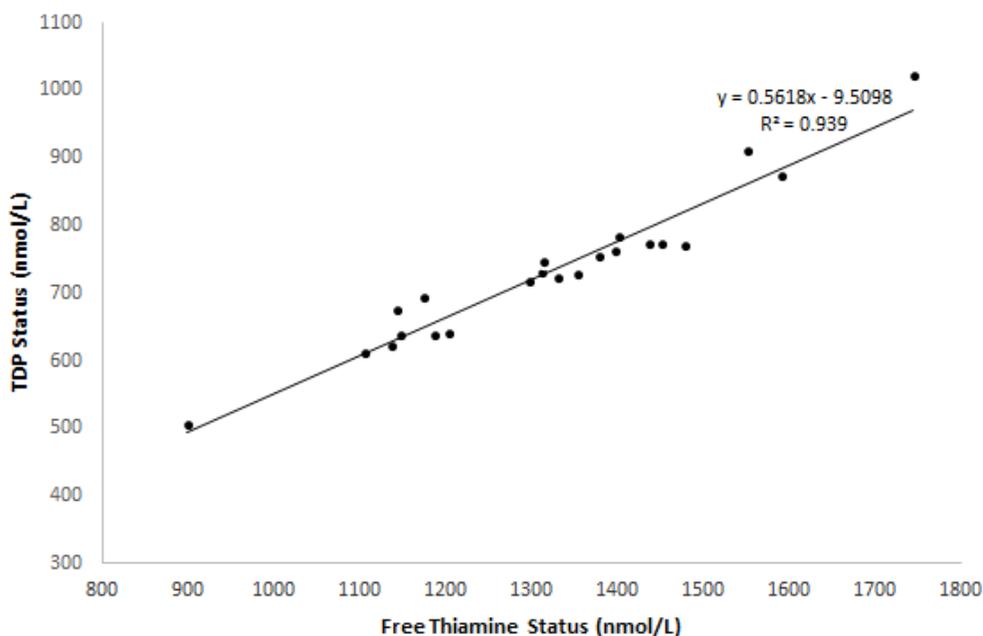


**Figure 3.1.** Relationship between age (in years) and thiamine diphosphate (TDP) status (nmol/L) in 22 healthy dogs. A significant negative relationship was observed ( $p = 0.01$ ).



**Figure 3.2.** Relationship between age (in years) and free thiamine status (nmol/L) in 22 healthy dogs. A significant negative relationship was observed ( $p = 0.02$ ).

Twenty healthy cats were enrolled in this study. Due to laboratory error in addition to a strong detector response from one form of thiamine masking the response of the second form on HPLC analysis (Appendix A), the free thiamine status of only one cat and the TDP status of eight cats could be determined. Thus, eight cats are included in study analysis and only TDP status is reported. Of the eight cats, six were Domestic Shorthairs and two were Siamese. Four cats were spayed females and four were neutered males. The average age of participants was  $6.1 \pm 3.3$  years. The average BCS and MCS upon study completion were  $5.6 \pm 0.9$  and  $2.9 \pm 0.4$ , respectively.



**Figure 3.3.** Relationship between free thiamine status (nmol/L) and thiamine diphosphate (TDP) status (nmol/L) in 22 healthy dogs. A significant positive relationship was present between the two forms of thiamine ( $p < 0.001$ ).

One cat received the chicken diet, three received the fish diet, and four received the poultry diet. The average daily thiamine intake was  $1.0 \pm 0.2$  mg/day as fed. The average TDP status for healthy cats was  $2041.3 \pm 808.9$  nmol/L (Table 3.1). There was no significant effect of age ( $p = 0.10$ ), sex ( $p = 0.31$ ), or

thiamine intake ( $p = 0.47$ ) on TDP status. There was insufficient variation in the data to assess the effect of diet type on TDP status.

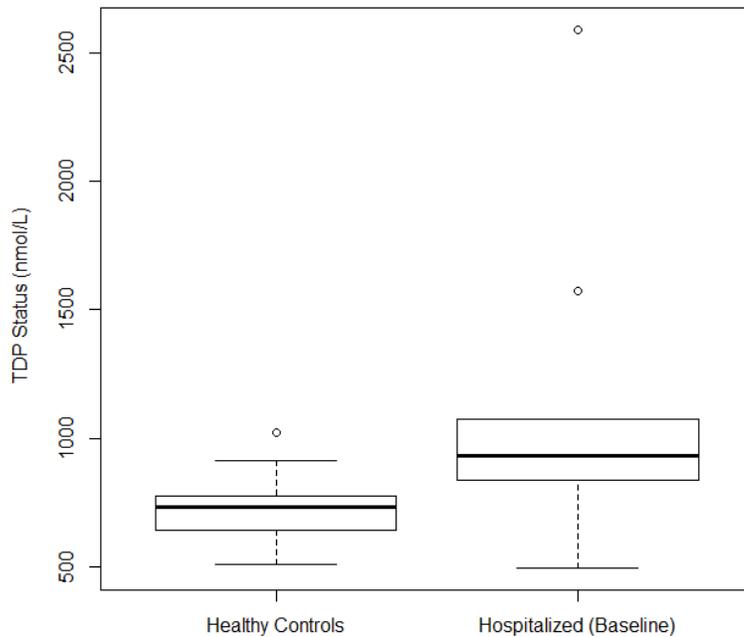
### Experiment 2 – Dogs and Cats with Reduced Appetite

A total of 12 dogs and three cats were enrolled in this experiment. Five dogs subsequently received thiamine supplementation. An insufficient number of cats were enrolled due to the smaller proportion of hospitalized cats meeting study requirements. Cats were therefore excluded from statistical analysis.

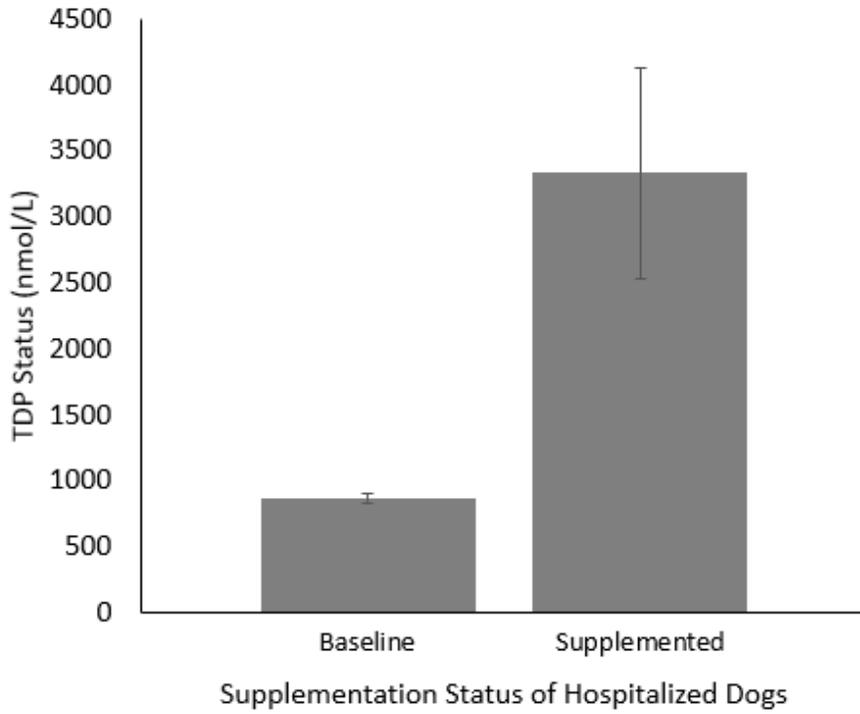
The baseline TDP status of 10 dogs and the baseline free thiamine status of five dogs were determined. Due to laboratory error and a strong detector response that masked one form of thiamine in a number of blood samples (Appendix A), TDP status could not be determined for two dogs, and free thiamine status could not be determined for seven dogs. For one dog (dog #4), neither baseline nor supplemented thiamine status (TDP or free thiamine) could be determined, and this dog was removed from all analyses. Post thiamine supplementation, TDP and free thiamine status were determined in three dogs. Only two dogs with post thiamine supplementation measurements had both a baseline and corresponding supplemented free thiamine status. Due to insufficient data, a comparison of free thiamine status before and after supplementation was therefore excluded from statistical analysis.

Participant information for inappetent dogs is presented in Appendix B. Median TDP status and free thiamine status of inappetent patients were 929.8 nmol/L (range: 493.5-2592.6) and 1461.9 nmol/L (range: 1319.8-2673.3), respectively. The TDP status of the subset of inappetent dogs that received supplementation was  $869.2 \pm 39.7$  nmol/L before supplementation and  $3331.0 \pm 799.2$  nmol/L after supplementation. Inappetent dogs had higher TDP status compared to healthy dogs from Experiment 1

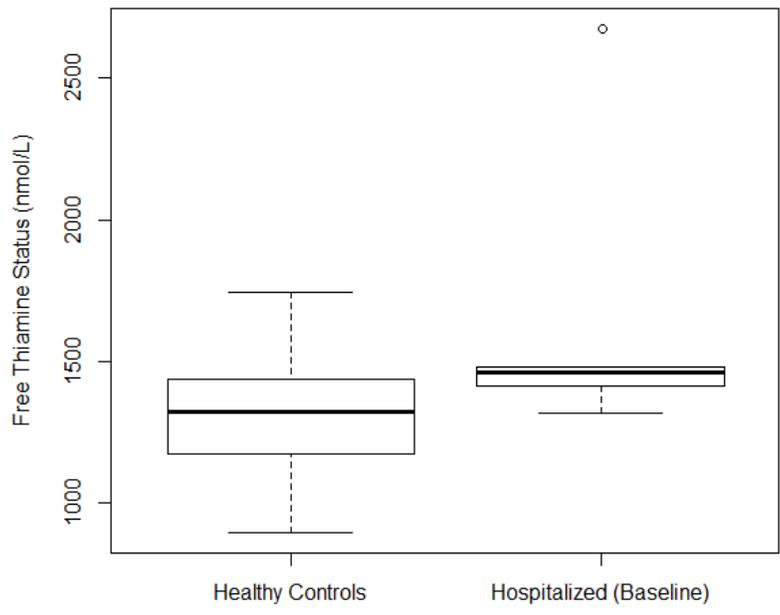
( $p = 0.001$ ; Figure 3.4), and TDP status was higher in inappetent dogs after thiamine supplementation ( $p = 0.04$ ; Figure 3.5). There was no significant difference between the free thiamine status of inappetent patients compared to that of dogs from Experiment 1 ( $p = 0.06$ ; Figure 3.6).



**Figure 3.4.** Comparison of thiamine diphosphate (TDP) status between 22 healthy control dogs and 10 hospitalized dogs with history of inappetence for at least 3 days. Inappetent dogs had significantly higher TDP status than healthy dogs ( $p = 0.001$ ).



**Figure 3.5.** Comparison of thiamine diphosphate (TDP) status (nmol/L) of 3 hospitalized dogs with a history of inappetence for at least 3 days, before and after receiving subcutaneous thiamine hydrochloride supplementation. There was a significant increase in TDP status of dogs following thiamine supplementation ( $p = 0.04$ ).



**Figure 3.6.** Free thiamine status (nmol/L) of 22 healthy dogs and 5 hospitalized dogs (baseline) with a history of inappetence for at least 3 days. There was no significant difference in free thiamine status between groups of dogs ( $p = 0.06$ ).

### 3.5 – Discussion

This study presents the first foray into free thiamine status in healthy dogs. To date, a reference range for thiamine status, either free thiamine or TDP, has yet to be determined in dogs and cats. One study examined TDP status in six dogs and reported a mean whole blood TDP status of 95  $\mu\text{g/L}$  (range: 84-104  $\mu\text{g/L}$ ) [17], while another found a median whole blood TDP concentration of 72  $\mu\text{g/L}$  (range: 46-112  $\mu\text{g/L}$ ) in dogs, and a median of 48  $\mu\text{g/L}$  (range: 20-90  $\mu\text{g/L}$ ) in cats [30]. Neither of these studies assessed dietary thiamine intake. Both studies used microbial methods to assess TDP status, where thiamine content is estimated based on growth of microbial organisms. However, there are limitations to this method [31], and it is no longer used with frequency. More recently, HPLC was used to determine a mean  $\pm$  s.d. whole blood TDP concentration of  $89.7 \pm 20.7$   $\mu\text{g/L}$  in dogs [32]. Free thiamine status has not been investigated in dogs or cats. In this study, TDP concentrations in RBCs of control dogs and cats were  $311.4 \pm 46.8$  and  $868.2 \pm 344.0$   $\mu\text{g/L}$ , respectively. The present study therefore found thiamine status in dogs and cats to be higher than previously reported. As TDP in blood is predominantly found within RBCs [2, 33], the present study used washed RBCs rather than whole blood, and hematocrits of blood samples were adjusted to ensure uniformity in results between samples. Differences in methods of analysis from previous studies could have led to the higher results seen in this study.

This study found a strong relationship between TDP status and free thiamine status in healthy dogs. This could have been a function of interconversion of TDP and free thiamine within the cytosol of RBCs. Since free thiamine is converted into TDP after it is taken up into RBCs [1], the strength of the relationship observed in this study suggests that a regulatory mechanism is in place to ensure that equivalent concentrations of each form of thiamine are present in RBCs.

The relative proportions of TDP and free thiamine in canine RBCs were unexpected. An estimated 80% of total thiamine in mammalian cells is phosphorylated, of which TDP is the most prevalent form [1, 2, 33]. However, in the present study, free thiamine had higher overall concentrations than TDP, suggesting that this form is more prevalent than TDP in canine RBCs. This result could be due to some unique characteristic of canine thiamine metabolism that differs from what is known about thiamine metabolism of humans. Variation as a result of the analysis methodology may also have contributed to these results. Further research on free thiamine status in dogs is therefore required to determine the cause of this apparent increase in unphosphorylated thiamine compared to TDP.

Three diets from a single manufacturer were fed to the healthy dogs in this study. Protein sources and thiamine concentrations differed in each diet, though neither diet nor daily thiamine intake had an effect on thiamine status of healthy dogs. The lack of effect of dietary thiamine content on TDP status is similar to previous findings in dogs [32], though this study did not specifically determine daily thiamine intake. Thiamine intake in cats also had no relationship with thiamine status, suggesting that dietary thiamine uptake is regulated in both species. This could occur within the intestine after ingestion [34], or it could be through the kidneys regulating the concentration of circulating blood thiamine after it has been absorbed through the intestine and excreting any excess [35, 36]. Indeed, in humans, urinary thiamine excretion is correlated with dietary thiamine intake [36]. In this study, urinary thiamine concentration was not assessed.

Recent meta-analyses of energy requirements in adult dogs and cats have found that there are similar metabolic requirements for dogs and cats of different sexes [37, 38], which could suggest that there may be no differential requirement for thiamine between males and females. However, neuter status may

also have an impact on results, as one canine participant in each study was intact. Intact dogs and cats have higher overall energy requirements than their neutered equivalents [37, 38], but the small population size of this study precluded an assessment of neuter status. It is presently unknown if intact animals require higher daily thiamine concentrations than neutered animals.

Changes in thiamine status, and in requirements for thiamine, based on animal age have not previously been assessed. In humans, elderly populations can have a high prevalence of thiamine deficiency [39], and may have poor thiamine status compared to middle-aged adults and compared to their own thiamine status from three years prior [40]. This decrease in thiamine status may be due to decreased intestinal absorption with age, though whether this effect is a result of age itself or age-related comorbidity is subject to debate [40, 41]. In this study, age had a significant negative impact on free thiamine and TDP status in dogs. This may be a result of age-related loss of intestinal absorptive ability, though further research is required to determine thiamine absorptive capacity in dogs of different ages. Geriatric cats have been found to have decreased intestinal absorption of some nutrients [42], although cats in the present study had no age-associated change in blood thiamine status. While it is possible that feline TDP status is unaffected by age or age-related factors, the small study population makes interpretation of results difficult.

In this study, hospitalized inappetent dogs had higher blood TDP concentrations compared to healthy dogs. A recent study found increased TDP status in dogs with chronic kidney disease compared to controls [43]. In this latter study, the authors hypothesized that decreased renal function could have led to poor thiamine excretion and consequent increased blood concentrations, though urinary losses were not examined in that study. In the present study, however, only two participants had been diagnosed with renal failure. Several patients had been diagnosed with gastrointestinal disorders such as pancreatitis, which in humans has been

associated with thiamine deficiency in medical case reports [44, 45]. The results of this study therefore suggest that differences may exist between human and canine thiamine metabolism, and that the relationships between certain diseases and thiamine deficiency between the two species may vary. Free thiamine status was not different between hospitalized and control dogs in this study, though interpretation of results was limited by small sample sizes. The increased TDP status in hospitalized dogs after supplementation is similar to results seen in hospitalized human patients [46] and healthy human adults [47]. In the present study, changes in thiamine status were not tracked between baseline and final blood collection periods, and the length of time required for canine blood TDP concentrations to reach a threshold level or decrease to a baseline concentration after supplementation was not assessed. A small study in humans suggests that plasma free thiamine status may return to baseline concentrations within 12h of IV supplementation, while phosphorylated thiamine status responds more slowly [47], but the authors did not provide statistical analyses nor assess diets of participants. In the present study, the impact of dietary thiamine intake in addition to supplementation was not assessed due to the small subset of dogs receiving supplementation. This is an area that requires further study to determine the efficacy of thiamine supplementation in combination with assisted/tube feeding in canine hospitalized patients.

Limitations are noted for both experiments in this study. In the first experiment, measurement of daily food intake may have been subject to inaccuracy, as measurement of extruded pet food using cups is imprecise [48]. Measurement of food intake using grams may be beneficial for future research. Additionally, both experiments were limited by small sample sizes. Fluctuations in the thiamine status of a small number of participants in both experiments could have led to large effects on results. The higher than expected thiamine values detected in some of the healthy dogs and cats, as well as the inappetent hospitalized dogs in this study, may reflect a true range of canine and feline blood thiamine concentrations, may represent

abnormal thiamine status in individual animals, or may be the result of variability in the HPLC methodology. In addition, thiamine status (either TDP or free thiamine) could not be determined for some participants due to difficulties with the HPLC analysis; thus, a comparison using both forms of thiamine could not be conducted for all participants.

When assessing thiamine levels in hospitalized inappetent animals, diet history and information regarding length of inappetence may have been incomplete for some participants due to imperfect owner recall. Additionally, as inappetence in dogs can occur from a variety of medical causes, the inclusion of dogs presenting with various diseases in this study means that it is difficult to extrapolate the relationship between a specific disease and an animal's blood thiamine status. This study's cross-sectional design also does not allow for causal conclusions, and it cannot be determined whether the thiamine status observed in participants upon study enrollment were a result of patients' diseases. In this study, each patient received several medications over the course of their hospitalization, including diuretics, antibiotics, and analgesics. However, while increased urinary thiamine loss as a consequence of diuretics has been well-examined [46, 49-51], the possible impact of other medications on thiamine status is unknown. Additionally, a comparison between the healthy and inappetent groups of dogs may provide limited clinical information due to the absence of matched healthy controls for hospitalized participants, and further research should therefore utilize matching of participants to better elucidate relationships between inappetent and healthy animals.

In conclusion, additional research is required to determine a physiological reference range for free thiamine and TDP status in adult dogs and cats, and to determine what range of concentrations is indicative of thiamine insufficiency or deficiency in both adult and senior dogs and cats. The relationship between free

thiamine and TDP status in dogs and cats also warrants further investigation to determine the relationship between thiamine metabolites. In inappetent animals, thiamine status of hospitalized dogs may not decrease within three days of inappetence, though suspicion of thiamine deficiency in a patient warrants supplementation with thiamine to resolve clinical signs. The relationship between diseases, medications, and thiamine status is poorly understood in dogs, and may not be comparable to human medical data. As supplementation was found to increase patients' thiamine status in a small subset of hospitalized dogs, further research will be beneficial to create thiamine supplementation protocols for diseases associated with thiamine deficiency. As inappetent cats could not be assessed in this study, further research is needed to examine thiamine status during disease, as well as the impact of supplementation, in hospitalized cats.

### **3.6 – Acknowledgements**

The authors thank the veterinary technicians at the Ontario Veterinary College (OVC) Central Animal Facility, Small Animal Clinic, and Intensive Care Unit for assistance with sample collection and supplement administration, as well as the clinicians at the OVC Small Animal Clinic for allowing us to enroll hospital patients in their care. The authors also thank Gabrielle Monteith and William Sears for statistical assistance.

### **3.7 – Footnotes**

- a. “1-Cup Vivid Colour Measure-Up.” 4imprint Canada, Ontario, Canada.
- b. Thiamine hydrochloride, 500mg/100mL. McCarthy and Sons Service, Alberta, Canada.
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### 3.8 – References

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## CHAPTER 4: GENERAL DISCUSSION

### 4.1 – General Conclusions of Experiments

#### 4.1.1 – Study 1: Thiamine Retention in Extruded Dog and Cat Food

It can be cost-effective for dog and cat owners to buy large bags of kibble and freeze the excess in an attempt to keep it fresh for longer periods of time. While thiamine degradation as a result of exposure to heat, oxygen, light, and alkaline pH are known [1-3], the effect of freezing on thiamine retention in foods has not specifically been examined, except in combination with other processing methods (e.g., canning, blanching) [4-7]. Thus, freezing of extruded pet food could pose a concern for ensuring sufficient dietary thiamine intake of dogs and cats. In this study, we observed no difference in thiamine concentrations of extruded dog and cat food after three months of storage at -20°C or at room temperature at approximately 24°C, suggesting that thiamine retention is equivalent at either storage temperature. In contrast, we found that the average thiamine concentrations among all diets increased over time and were significantly higher after three months of storage compared to five weeks.

These results were surprising, as past research assessing thiamine's poor retention during cooking and storage suggested that a decrease in thiamine concentrations would be seen in this study. Additionally, a previous study found that thiamine content in extruded dog and cat food decreases after 18 months, though storage information was not specified for the diets [8]. Extruded ruminant feed also undergoes thiamine losses during storage [9]. As Study 1 only ran for three months, there may not have been sufficient time for a noticeable effect on thiamine content to be seen. Future research should examine thiamine concentrations over a longer period of time (e.g., 12 months) to determine longer-term effects of storage temperature on dietary thiamine content.

In hindsight, an assessment of the moisture content of each sample would have provided valuable information, and may have helped determine if fluctuations in moisture could have led to the observed increase in thiamine concentrations over time. Moisture losses have been noted during storage of food products [10, 11], and while all study diets were stored in airtight bags to minimize exposure to air, some moisture loss may have occurred. Additionally, thiamine content of extruded pet food has historically been difficult to accurately determine [12]. Thus, since thiamine concentrations in the present study were not assessed using replicates, results may have been influenced by variation due to the analysis methodology.

It should be noted that all dog and cat foods in this study, throughout the entirety of the study period, exceeded their respective canine and feline AAFCO 2017 minimum dietary recommendations for thiamine [13]. In extruded dog and cat foods containing sufficient thiamine upon manufacturing, potential changes in thiamine content after three months of storage therefore may not pose a significant clinical concern with respect to providing adequate dietary thiamine to dogs and cats.

#### **4.1.2 – Study 2: Thiamine Status in Dogs and Cats**

In the first phase of Study 2, red blood cell (RBC) thiamine concentrations (thiamine diphosphate (TDP) and free thiamine) were analyzed in a group of healthy, client-owned dogs and cats being fed the diets described in Study 1. Unfortunately, only TDP status could be assessed in cats, and free thiamine status of cats therefore requires further investigation.

While free thiamine status has not previously been reported in dogs or cats, our study found TDP concentrations in both species to be higher than what has been found by other researchers [14-16]. Methodological differences may have had a role, such as evaluating thiamine content in RBCs rather than whole blood, but the reason for this difference was not examined. However, the lack of similarity between our results and the results of prior studies suggests that more information is needed before a reference range for thiamine status in dogs and cats can be determined. Another unexpected finding involved the relative proportions of free thiamine and TDP in canine blood, which are contrary to what has been presented in previous research. Free thiamine is present in human blood at much lower concentrations compared to TDP, which is the most prevalent form of thiamine [1, 17]. However, in Study 2, free thiamine had an overall higher concentration than TDP. It is therefore possible that thiamine metabolism in dogs differs from what is known in humans, but the mechanism and extent of any species-specific metabolic responses have not been examined.

Interestingly, we found that canine TDP and free thiamine status both decreased with increasing participant age. In humans, some research suggests that TDP status decreases with age [18], but not all studies have found an age-related effect [19]. However, elderly populations can have high incidences of thiamine deficiency [20]. Inefficient thiamine absorption may be related to the lower thiamine status seen in aging individuals, but more research is required to determine if this decline is due to metabolic changes or comorbidity. In our study, age did not have an effect on TDP status in cats, though the small feline sample size limited our ability to interpret results.

Differences in diet had no effect on resultant thiamine status in dogs, which is in line with previous findings [14]. However, no studies have specifically assessed the relationship between daily thiamine

intake and thiamine status in either dogs or cats. The relationship between thiamine intake and status in cats was not significantly related in Study 2, yet due to the small number of cats enrolled in this study, the effect of specific diets on TDP status could not be assessed. In both dogs and cats in Study 2, male and female participants had similar thiamine statuses, though sex has not previously been assessed in relation to thiamine status in either dogs or cats.

The second phase of this study compared thiamine status of dogs from the first phase to dogs presenting to the Ontario Veterinary College with a history of inappetence. As thiamine is an essential dietary nutrient in dogs [21], we expected hospitalized patients to have lower thiamine status compared to healthy dogs. Surprisingly, TDP status of hospitalized dogs was higher than that of healthy dogs. However, as was expected, supplementation with thiamine led to a significant increase in TDP status in hospitalized dogs compared to their pre-supplemented blood concentrations. While free thiamine status was not significantly different between healthy and inappetent dogs, baseline free thiamine status could only be determined for five inappetent dogs and only two inappetent dogs had post-supplementation free thiamine status. Results are therefore limited, and the effect of inappetence and supplementation on free thiamine status would benefit from additional research.

One study has reported that renal disease is associated with elevated TDP status in dogs compared to controls [22], which is in contrast to human research that shows a decrease in TDP status in patients with chronic kidney disease [23]. However, only two inappetent dogs in our study were diagnosed with renal disease. Thus, it is possible that other diseases may impact thiamine status in dogs, and further research is needed to determine whether illnesses other than renal disease affect thiamine status in dogs. In addition, we did not have a large enough sample size in either phase of this study to compare thiamine status of

inappetent dogs to matched controls; thus, the thiamine status of healthy dogs may not be perfectly comparable to that of the inappetent dogs.

Laboratory error led to the invalidation of blood samples from 11 healthy cats, and poor readability of results via high-performance liquid chromatography (HPLC) led to only one form of thiamine being detected for certain study participants, which impacted our ability to interpret results for study participants. However, the reason for these poor analysis results in only some participants, and not others, remains unknown.

## **4.2 – Future Directions**

### **4.2.1 – Study 1: Thiamine Retention in Extruded Dog and Cat Food**

The effect of thiamine-destroying compounds (thiaminases) on thiamine degradation in extruded kibble is an area for future research. Thiaminases are enzymes present in raw fish, and some shellfish and bacterial species, that can degrade thiamine [1]. While thiaminases denature when exposed to high heat [24, 25], such as the high heats utilized during extrusion cooking of pet food [26], the inclusion of raw fish as an ingredient in pet foods could lead to thiamine degradation if all ingredients are mixed prior to cooking. Similarly, variability in the extrusion process itself (e.g., fluctuations in cooking temperature) can impact the degree of thiamine loss [26]. Thus, the differential thiamine losses in finished kibble products as a result of extrusion are an area that can benefit from further research. Additionally, as extruded dog and cat foods can be found in a variety of packaging styles, the utilization of different containers and packaging materials on thiamine degradation warrants further investigation to determine an optimal storage medium that will minimize thiamine degradation, as well as potential degradation of other nutrients, in pet foods.

#### **4.2.2 – Study 2: Thiamine Status in Dogs and Cats**

Thiamine metabolism in dogs and cats needs further study to determine species-specific characteristics of absorption or utilization. For example, cats require approximately four times more dietary thiamine than dogs [21], but the reason behind their increased metabolic requirement is unknown and requires further study. Additionally, thiamine has a role in carbohydrate metabolism as a cofactor in the Krebs cycle [1], and diets high in protein and fat therefore have a thiamine-sparing effect due to the decreased demand for thiamine to break down carbohydrates in these diets. Thus, the effect on thiamine status of diets with varied compositions of macronutrients may provide valuable information on thiamine metabolism in dogs and cats. Similarly, the ability of the intestine to absorb thiamine in different forms (e.g., thiamine hydrochloride, thiamine mononitrate) may provide information that can be used by pet food manufacturers to ensure maximal bioavailability of thiamine supplements added to commercial dog and cat foods.

To date, thiamine status of healthy dogs and cats has been poorly studied. Diagnosis of thiamine deficiency presently relies on identification of clinical signs and risk factors (e.g., consumption of an unbalanced diet), and diagnosis is confirmed if signs resolve after administration of a thiamine supplement. Development of a reference range may therefore provide an additional clinical tool in confirming thiamine deficiency.

Additionally, more knowledge is required to examine the effect of disease processes on thiamine status in dogs and cats, as there may be unique relationships between a given illness and thiamine metabolism in these species. Similarly, while the association between diuretic usage and increased thiamine excretion is well-examined in humans and rats [27, 28], other medications may have an as-yet unknown impact on thiamine absorption or excretion in dogs and cats.

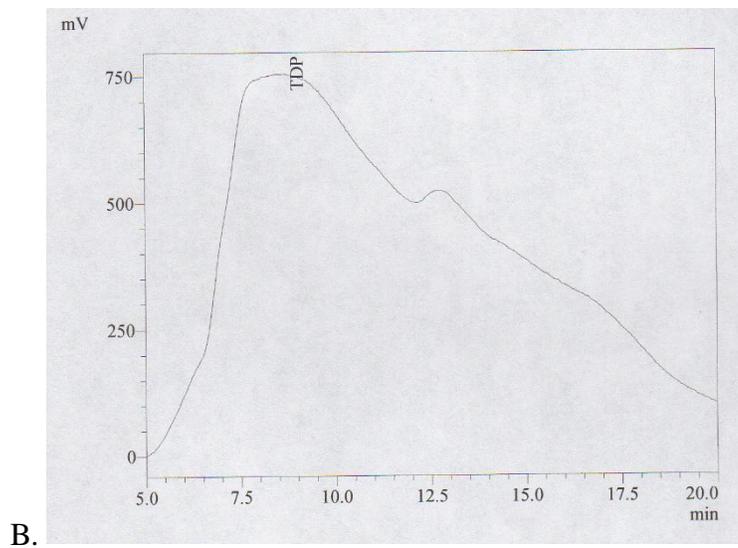
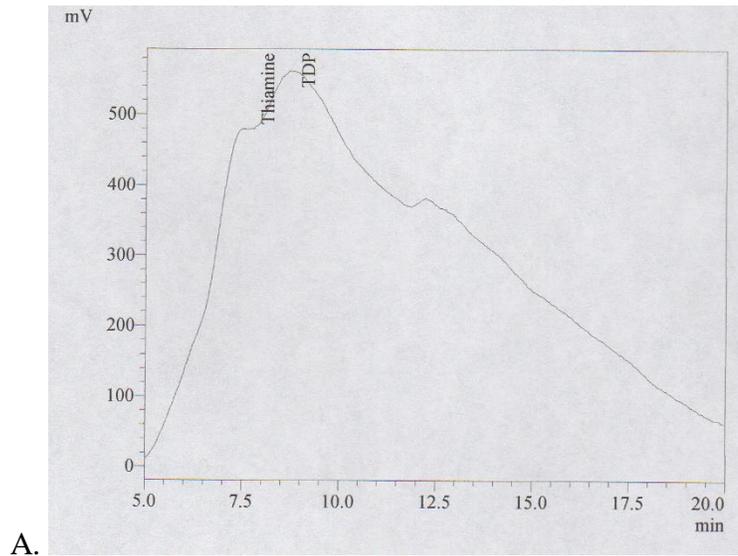
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## Appendix A

Example of chromatogram output from high-performance liquid chromatography (HPLC) analysis of free thiamine and thiamine diphosphate (TDP) status in blood of dogs and cats. A) shows a chromatogram where both free thiamine and TDP were detected, while in B) there was a strong detector response for TDP that masked the response for free thiamine, and did not permit for free thiamine to be detected.



## Appendix B

Participant data for inappetent canine study participants hospitalized at the Ontario Veterinary College Health Science Centre's Intensive Care Unit.

Participant #	Breed	Age (yrs)	Sex	BCS (/9)	MCS (/3)	Primary Diagnosis	Free Thiamine Status (nmol/L)		TDP Status (nmol/L)	
							Baseline	Suppl.	Baseline	Suppl.
1	German Shepherd	8	FS	3	1	Bite wounds	1319.8	NA	808.5	NA
2	Miniature Pinscher	8	FS	7	3	Diabetic ketoacidosis	1412.2	6456.5	835.5	3871.4
3	Miniature Schnauzer	6	FS	8	3	Pancreatitis	1461.9	6440.3	859.2	3708.6
5	Bernese Mountain Dog	5	FS	3	3	Renal disease	2673.3	NA	1572.5	NA
6	Maltipoo	8	FS	5	2	Renal disease	NA	NA	1069.7	NA
7	Bulldog	11	FS	3	1	Lymphoplasmacytic gastritis, pancreatitis	NA	NA	913.0	2412.9
8	Miniature Dachshund	12	FS	3	1	Protein-losing enteropathy	NA	NA	2592.6	NA
9	Dachshund	8.5	FS	5	3	Lymphangiectasa, gastric ulcer	NA	NA	493.5	NA
10	Boxer	7.5	MN	5	1	Hypercalcemia of unknown etiology	1481.7	NA	NA	NA
11	Yorkshire Terrier	12	F	4	3	Cholangiohepatitis	NA	NA	1073.0	NA
12	German Shepherd	13	FS	4	2	Pancreatitis	NA	NA	946.5	NA

NA – result not available

BCS – body condition score, MCS – muscle condition score, F – intact female, FS – spayed female, MN – neutered male