Universidade de Trás-os-Montes e Alto Douro

Urinary pH manipulation - Effects of diets and supplements on the urinary pH in dogs

– Versão Definitiva –

Dissertação de Mestrado Integrado em Medicina Veterinária

Inês Alcaide Igreja

Orientadora
Professora Doutora Ana Luísa Dias Guimarães Lourenço

Coorientador
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Vila Real, 2020

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Urinary pH manipulation - Effects of diets and supplements on the urinary pH in dogs

ORIENTADORES:
Professora Doutora Ana Luísa Dias Guimarães Lourenço
Professor Doutor Ronald Jan Corbee

ANO DE CONCLUSÃO: 2020

Declaro que esta Dissertação de Mestrado é resultado da minha pesquisa e trabalho pessoal, e das orientações dos meus supervisores. O seu conteúdo é original, e todas as fontes consultadas estão devidamente mencionadas no texto e na bibliografia final. Declaro ainda que este trabalho não foi apresentado em nenhuma outra instituição para obtenção de qualquer grau académico.

Vila Real, 2020

"In food excellent medicine can be found, in food bad medicine can be found; good and bad are relative."
Hippocrates

"Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less."
Marie Curie

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ABSTRACT

The urinary pH is a valuable and easily measurable biochemical marker and a vital part of the urinalysis as it is essential for the interpretation of urine chemistry and may reflect systemic acid-base abnormalities. In patients with uroliths, it can be used to determine the need for urinary pH manipulation and help monitor responses to treatment.

In the first part of this dissertation a review on the current knowledge about urinary pH and his variation during the day, urinary pH manipulation using oral supplementation and the methods used in the measurement of this parameter, is presented.

In the practical section of this dissertation a study was carried out whose objectives were to: 1) evaluate the influence of two therapeutic diets formulated to prevent the incidence of uroliths, Hill's® Prescription Diet® u/d® Canine (u/d) and Royal Canin Urinary SO dog (Urinary SO) on urinary pH; 2) to evaluate the effect of oral supplementation of potassium citrate and an oral solution containing ammonium chloride (Urical) in the urinary pH; 3) evaluate the effect of the postprandial alkaline tide in the urinary pH of dogs. The following hypothesis were presented: 1) u/d diet (MER = 1.6 x 70 $BW^{0.75}$), fed over two meals per day, or potassium citrate (130-211 mg/kg BW/day), given two times daily with food, causes a significant increase ($P<0.05$) in the dog's urinary pH when compared with the control; 2) Urinary S/O diet (MER = 1.6 x 70 $BW^{0.75}$), fed over two meals per day, or Urical (5 ml/10kg BW/day), given 2 times daily with food, causes a significant decrease ($P<0.05$) in the dog's urinary pH when compared with the control ; 3) Food intake causes a transitory increase in pH (alkaline tide).

The study lasted 31 days. During this period, seven dogs participated in four different trials, in order to assess the effect of each treatment. Each trial lasted 2 to 5 days and was interrupted by a washout interval of 2 to 4 days. Urinary pH measurements were performed every two hours between 07h00 and 15h00, with the food being given at 07h00 and 15h00, right after the measurements. The effect of each treatment was evaluated by comparison with a group fed a maintenance adult dog diet.

The u/d diet and the potassium citrate caused a significant increase ($P<0.05$) in urinary pH. The urinary SO diet caused a significant decrease ($P<0.05$) in urinary pH, however, Urical did not cause a significant decrease ($P>0.05$) in urinary pH. Food intake caused a transient increase in urinary pH between 09h00 and 11h00 (approximately 2-4 hours after intake).

The present study confirms that nutrition does influence acid-base balance in dogs, thus food can be a valuable tool to manipulate pH. The influence of ingredients/diets on urinary pH in dogs should be further investigated, so that urinary pH control can be effective and predictable.

Key words: Urinary pH, Acidifiers, Alkalizers, Nutritional supplements, Clinical Nutrition, Dogs.

RESUMO

O pH urinário é um marcador bioquímico valioso e facilmente mensurável. Constitui uma variável indispensável no exame de urina, pois é essencial para a interpretação dos resultados da bioquímica, podendo refletir alterações ácido-base sistémicas. Constitui uma ferramenta bastante útil, em doentes com urólitos, podendo ser usado para determinar a necessidade de manipulação do pH urinário e auxiliar na monitorização da eficácia do tratamento da doença.

A primeira parte desta dissertação apresenta uma revisão bibliográfica sobre o pH urinário, que inclui a sua manipulação recorrendo a suplementos orais e os métodos utilizados para a medição desta variável.

Na parte prática desta dissertação foi realizado um estudo com os objetivos de: 1) avaliar a influência de duas dietas terapêuticas formuladas para prevenir a formação de úrolitos, Hill's® Prescription Diet® u/d® Canine (u/d) e Royal Canin Urinary SO dog (Urinary SO), no pH urinário; 2) avaliar o efeito da suplementação oral com citrato de potássio e com uma solução oral contendo cloreto de amónio (Urical) no pH urinário; e, 3) avaliar o efeito da maré alcalina pós-prandial no pH da urina em cães. As hipóteses colocadas foram as seguintes: 1) a dieta u/d (MER=1,6 x 70 $BW^{0,75}$), distribuída em duas refeições por dia, ou o citrato de potássio (130-211 mg/kg de BW/dia), administrado duas vezes por dia com o alimento, provoca um aumento significativo ($P<0,05$) no pH urinário do cão em comparação com o controlo; 2) a Urinary SO (MER=1,6 x 70 $BW^{0,75}$), distribuída em duas refeições por dia, ou Urical (5 ml/10kg de BW/dia), administrado duas vezes por dia com o alimento, provoca uma diminuição significativa ($P<0,05$) no pH urinário do cão em comparação com o controlo; 3) A ingestão de alimento provoca um aumento transitório do pH (maré alcalina).

O estudo teve a duração de 31 dias. Durante este período, sete cães participaram em quatro ensaios diferentes, de modo a avaliar o efeito de cada um dos tratamentos. Cada ensaio teve uma duração de 2 a 5 dias com 2 a 4 dias de intervalo entre si. Foram realizadas medições do pH urinário a cada duas horas entre as 07h00 e as 15h00, sendo que as refeições foram oferecidas às 07h00 e 15h00, logo a seguir às medições. O efeito de cada tratamento foi avaliado por comparação com um grupo alimentado com uma dieta para cão adulto em manutenção.

A dieta u/d e o citrato de potássio provocaram um aumento significativo ($P<0,05$) no pH urinário. A dieta Urinary SO provocou uma diminuição significativa ($P<0,05$) no pH urinário, no entanto, a solução Urical não provocou alteração significativa ($P>0,05$) no pH urinário. A

ingestão de alimento provocou uma maré alcalina, identificada pelo aumento transitório no pH urinário entre as 9h00 e 11h00 (aproximadamente 2-4 horas após ingestão).
Este estudo confirma que a nutrição influencia o equilíbrio ácido-base em cães, constituindo assim uma ferramenta valiosa na manipulação do pH urinário. A influência de ingredientes/dietas sobre o pH urinário em cães é uma área a investigar, para que seja possível o seu controlo efetivo e previsível.

Palavras-chave: pH urinário, Acidificantes, Alcalinizantes, Suplementos nutricionais, Nutrição Clínica, Cães.

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List of Acronyms, Abbreviations and Symbols

ATP – Adenosine triphosphate

BBS – Bicarbonate Buffer System

BID – *bis in die;* twice a day

BW – Body weight

Ca^{2+} – Calcium ion

Cl^- – Chloride ion

CO_2 – Carbon dioxide

DM – Dry Matter

ECF – Extracellular fluid

GI – Gastrointestinal

h – hours

H^+ – Hydrogen ion

HCO_3- – Bicarbonate ion

ICF – Intracellular fluid

IM – Intramuscular

IV – Intravenous

K^+ – Potassium ion

Kcal – Kilocalorie

MER – Maintenance Energy Requirement

Mg^{2+} – Magnesium ion

Na^+ – Sodium ion

NH_4^+ – Ammonium ions

NH_4Cl – Ammonium chloride

OH^- – Oxygen hydroxide

P – Phosphorus

PCO_2 – Partial pressure of CO_2

PO – *per os*

PTH – Parathyroid hormone

RTA – Renal Tubular Acidosis

SC – Subcutaneous

SD – Standard Deviation

UPC – Urine Protein:Creatinine ratio

USG – Urine Specific Gravity

Chapter I

Introduction

1. Urinary pH

Urinary pH is a rough but helpful estimate of acid-base balance (Barsanti, 2012). Urinary pH is not constant and may fluctuate over the 24 hours. There is an indication of a physiologic acid-base rhythm with a curve of diurnal fluctuation which is characteristic for a given individual, and it is a result of several factors such as diet, emotional status, exercise, personality and pulmonary ventilation (Elliot, Sharp and Lewis, 1959). The physiological process regarding the alkalization of the blood and urine, *i.e.* rise in the pH, following a meal is designated the alkaline tide effect (Niv and Fraser, 2002).

2. Urinary pH manipulation

Food and endogenous metabolic processes are the sources of acid or base intake or production, and so it is possible to efficiently alter or adjust the urinary pH by solely dietetic means (Dwyer *et al.*, 1985; Remer and Manz, 1994). Urinary pH manipulation is beneficial in multiple conditions but is particularly important in some types of urolith formation, considering urine acidity or alkalinity influences what type of mineral precipitates (Gleaton, Bartges and Laflamme, 2001). Numerous therapeutic diets are formulated to prevent recurrence of uroliths in dogs. The potential of a diet to acidify/alkalize the urine depends on its ingredients and the equilibrium between acidifiers, such as methionine, calcium sulfate and ammonium chloride, or alkalizers, such as calcium carbonate and potassium citrate (Queau, 2019).

3. Objectives

The main goal of this work was to review the current knowledge on urinary pH and his variation during the day, urinary pH manipulation using oral supplementation and the methods used in the measurement of this parameter. Furthermore, compare it to a clinical research project performed on a Referral Small Animal Hospital, by assessing the effect of diets and supplements on the urinary pH while also evaluating his diurnal fluctuations in dogs.

Chapter II

State of the art

1. Acid-base balance

The term pH is designated as the negative base 10 logarithm of the hydrogen ion concentration (H^+) expressed in mol per liter, or the base 10 logarithm of the reciprocal of the hydrogen ion concentration, its average value in plasma is 7.407 (7.351-7.463) and 7.386 (7.310-7.462) in dogs and cats, respectively (Chew and DiBartola, 1998; DiBartola, 2012; Humm, 2012). There is an inverse relationship between pH and H^+: the higher the H^+, the lower the pH. Also, pH and H^+ do not vary linearly with one another but exponentially. H^+ is the smallest ion with an atomic weight of one (DiBartola, 2012). Nonetheless, H^+ ions are very powerful because they are intimately involved in the capture of energy from the oxidation of fuels by managing the regeneration of ATP (Guyton and Hall, 2011; Koeppen and Stanton, 2013; VanPutte, 2016).

The pH of a solution is dependent on the difference between the numbers of negatively and positively charged particles present in the solution. If positively charged particles are added to a solution, such as the plasma, the number of H^+ cations decreases and the number of oxygen hydroxide (OH^-) anions increases in order to maintain the electro-neutrality of the solution (the solution becomes more alkaline). Conversely, adding anions to a solution causes an increase in H^+ and a decline in OH^-, in order to maintain electro-neutrality, and the pH of the solution decreases (DiBartola, 2012; Koeppen and Stanton, 2013; Kamel and Halperin, 2017).

The pH, and ergo the $[H^+]$, alters when either the bicarbonate anion (HCO_3^-) concentration or the partial pressure of CO_2 (PCO_2) is altered (an increase in PCO_2 produces acidosis, whereas a decrease in PCO_2 produces alkalosis). Acid-base balance disturbances that emerge from a $[HCO_3^-]$ change are named metabolic acid-base disorders when those that emerge from a change in the PCO_2 are named respiratory acid-base disorders (Koeppen and Stanton, 2013).

The kidneys are primarily accountable for regulating the $[HCO_3^-]$ of the extracellular fluid (ECF), and the lungs control the PCO_2. The concentration of H^+ in the body fluids is low compared with that of other ions and must be maintained in a tight range. If their concentration rises, H^+ ions will bind to intracellular proteins, and this changes their charge, shape, and possibly their functions. Consequently, a system is needed to remove H^+ ions, even if their

concentration is not significantly elevated (DiBartola, 2012; Koeppen and Stanton, 2013; Kamel and Halperin, 2017).

There are three primary systems for regulation of H^+ concentration in the body fluids to prevent acidosis or alkalosis. These mechanisms are the following: buffer systems, the respiratory system, and the kidneys (Allen, 1996; Guyton and Hall, 2011; VanPutte, 2016).

Buffer systems

A buffer is a compound that can accept or donate protons (hydrogen ions) and minimizes a change in pH (Guyton and Hall, 2011; VanPutte, 2016).

Three critical buffer systems regulate significant fluctuations in the pH of body fluids. These are the bicarbonate buffer system (BBS), the phosphate buffer system, and the protein buffer system (VanPutte, 2016).

Body buffers can be classified as bicarbonate and non-bicarbonate buffers (protein and phosphate buffers). The bicarbonate buffer system is the main buffer system of ECF, since it plays a crucial part in the regulation of the extracellular pH (DiBartola, 2012; VanPutte, 2016). The BBS plays a fundamental part in the acid-base balance by both the respiratory system and the kidneys, despite having a limited ability to oppose changes in pH. It differs from the other buffer systems of the body (*e.g.*, phosphate) because it is regulated by both the lungs and the kidneys (VanPutte, 2016). The BBS counters a significant decrease in pH when acidic substances are added to a solution. It also responds to the addition of basic substances resisting a substantial increase in pH (DiBartola, 2012). When a strong acid (*e.g.,* HCl) is added to the bicarbonate buffer solution, the increased H^+ released is buffered by HCO_3. Subsequently, a very weak acid H_2CO_3 is formed, which consecutively forms CO_2 and H_2O. This reaction progresses slowly, but the enzyme carbonic anhydrase significantly increases its rate. The excess CO_2 is eliminated from the EFC by stimulating respiration. If a strong base is added to the bicarbonate buffer solution, the opposite reactions occur (Guyton and Hall, 2011).

The phosphate buffer system is an essential intracellular buffer system because the concentration of phosphate in this fluid is much more significant than in the extracellular fluid (Guyton and Hall, 2011; VanPutte, 2016). In opposition to its somewhat irrelevant role as an extracellular buffer, the phosphate buffer is particularly vital in the tubular fluids of the kidneys, considering phosphate usually becomes significantly concentrated in the tubules (Guyton and Hall, 2011). Ions, such as HPO_4^{2-}, bind H^+ to form $H_2PO_4^-$, when the pH becomes more acidic,

on the contrary, H_2PO_4 releases H^+ into solution, when the pH becomes more alkaline (VanPutte, 2016).

Proteins are between the most abundant buffers in the body because of their significant concentrations, especially within the cells (Guyton and Hall, 2011; Kamel and Halperin, 2017). The ability of proteins to function as buffers are conferred by the functional groups of amino acids (carboxyl or amino groups); they have the capacity to serve as weak acids and bases. Therefore, when H^+ concentration increases, more H^+ binds to the functional groups, and contrarily H^+ is freed from the functional groups when H^+ concentration decreases (DiBartola, 2012; VanPutte, 2016).

Buffers almost instantaneously combine with acid or base to prevent excessive H^+ concentration changes, *e.g.*, a small increase in H^+ ion concentration in the plasma will quickly titrate bicarbonate ions in ECF and then titrate intracellular buffers, but the regulation of respiration and the function of the kidneys also perform essential tasks (Guyton and Hall, 2011; VanPutte, 2016).

Respiratory system

The respiratory system responds within a few minutes to bring the pH of body fluids back toward its normal range (Guyton and Hall, 2011; VanPutte, 2016). The lungs maintain the blood pH within a narrow range by adjusting the rate at which CO_2 is excreted in relationship to the actual alteration in the blood bicarbonate level, respiratory compensation (Rune and Lassen, 1968; DiBartola, 2012). The stimulation of the respiratory center causes hyperventilation, resulting in PCO_2 decreasing to below normal. This response, which begins immediately and is completed within hours, minimizes the change in pH because the ratio of HCO_3- to PCO_2 is normalized (Koeppen and Stanton, 2013; Kamel and Halperin, 2017). Even though the lungs can sustain or adjust pH by changing the PCO_2, this process can't generate either loss or gain in hydrogen ions (Remer, 2000).

Renal system

Although the kidneys are comparatively slow to respond, over hours to several days, they are the most potent of the acid-base regulatory systems (Guyton and Hall, 2011; DiBartola, 2012; VanPutte, 2016). The kidneys regulate acid-base balance by excreting either acidic or alkaline urine. Excreting acidic urine reduces the amount of acid in ECF, while excreting alkaline urine removes base from the ECF (Remer, 2000; DiBartola, 2012; Koeppen and Stanton, 2013).

Large amounts of HCO_3^- are filtered continuously within the tubules, and if they are excreted into the urine, this removes base from the blood. Large amounts of H^+ are also secreted into the tubular lumen by the tubular epithelial cells, therefore removing acid from the blood. If more H^+ is secreted than HCO_3^- is filtered, there will be a net loss of acid from the extracellular fluid. Conversely, if more HCO_3^- is filtered than H^+ is secreted, there will be a net loss of base (Allen, 1996; DiBartola, 2012; Kamel and Halperin, 2017).

Removal of H^+ ions by the BBS leads to a shortage of HCO_3- ions. Hence, one must have another system that adds new HCO_3^- ions to the body as long as acidosis persists. The regeneration of lost bicarbonate is a critical task of the kidneys, in the metabolic process of excretion of ammonium ions (NH_4^+) in the urine (DiBartola, 2012; Koeppen and Stanton, 2013; Kamel and Halperin, 2017). Although all of the plasma bicarbonate is filtered in the glomerulus, virtually all is reabsorbed back into the blood. Most of this reabsorption happens in the proximal convoluted tubule (Allen, 1996; Koeppen, 2009).

The amount of reabsorbed bicarbonate is regulated via several mechanisms in response to changes in blood pH. It increases during acid loads and decreases during alkali loads.

While the proximal tubule returns filtered bicarbonate back to blood, the collecting duct generates new bicarbonate by actively secreting acids. As protons are depleted from the distal tubule cells, more bicarbonate is produced, which then exit into the blood (Allen, 1996; VanPutte, 2016).

In the renal tubule lumen, hydrogen ions and bicarbonate combine to form carbonic acid. Then carbonic acid splits up, catalyzed by membrane-bound carbonic anhydrase, in water and carbonic dioxide inside the tubule cells, and it dissociates back to protons and bicarbonate. The bicarbonate is then reabsorbed back into the blood, and the hydrogen ions secreted into the lumen. Bicarbonate reabsorption occurs primarily via a sodium symporter (Guyton and Hall, 2011; DiBartola, 2012; Koeppen and Stanton, 2013). There is also evidence that some HCO_3- exits the tubule cell across the basolateral membrane to the blood, in exchange for chloride (Koeppen, 2009). The hydrogen ions are either actively secreted by H^+-ATPase or exchanged for sodium from the filtrate. Hydrogen ions secreted into the lumen combine with urinary buffers, mainly filtered phosphate and ammonia, to be excreted in the urine (Koeppen, 2009; Guyton and Hall, 2011; DiBartola, 2012). The kidney regulation of body fluid acid-base balance is presented in **Figure 1**.

Other weak buffer systems, such as urate and citrate, are much less critical. The ammonia buffering system is particularly important because unlike phosphate, which is filtered in fixed

amounts from the plasma and can be depleted during high acid loads, ammonia production is regulated by changes in acidity and its concentration may increase several folds if necessary (DiBartola, 2012; Koeppen and Stanton, 2013; VanPutte. *et al.*, 2016).

There are two forms of ammonia in aqueous solution, the non-ionized NH_3 and the monovalent cation NH_4^+. The non-ionized form is lipid-soluble and easily crosses cell membranes in the direction determined by the concentration gradient. On the contrary, the NH_4^+ is trapped within the tubular lumen and is excreted in the form of neutral salts, like NH_4Cl (Allen, 1996; Remer, 2000; Koeppen, 2009; DiBartola, 2012).

Ammonia is produced from glutamine metabolism, primarily in the proximal tubule cells, although there is evidence for ammoniagenesis by most renal epithelial cells (Weiner and Verlander, 2013). With chronic metabolic acidosis, the production of ammonia is increased, and so is the urinary ammonium ion concentration (Allen, 1996; Koeppen and Stanton, 2013). It is consequently helping to preserve systemic acid-base balance, not only by the generation of HCO_3^- but also because it promotes acid excretion. It is suggested that cats do not react to metabolic acidosis like other species (Allen, 1996; Weiner and Verlander, 2013). They do not seem to increase the production of ammonia and glucose from glutamine throughout metabolic acidosis (Allen, 1996).

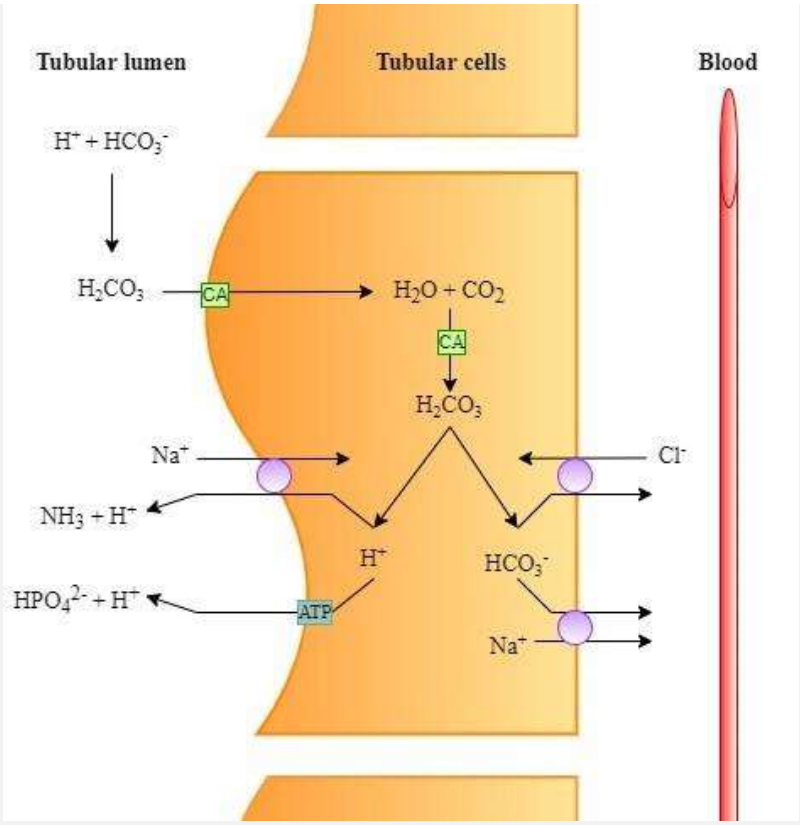


Figure 1: Kidney regulation of body fluid acid-base balance. Hydrogen ions (H^+) and bicarbonate (HCO_3^-), combine to form carbonic acid (H_2CO_3). Then carbonic acid splits up, catalyzed by carbonic anhydrase (CA), in water (H_2O) and carbonic dioxide (CO_2), and it dissociates back to protons and bicarbonate. The bicarbonate is then reabsorbed back into the blood, primarily via a sodium (Na^+) symporter or in exchange for chloride (Cl^-). The hydrogen ions are either actively secreted by H+-ATPase or exchanged for sodium. Hydrogen ions combine with phosphate (HPO_4^{2-}) and ammonia (NH_3), to be excreted in the urine. Adapted from: (Koeppen, 2009; Guyton and Hall, 2011; VanPutte. et al., 2016).

Every day, acid and alkali are ingested in the diet. Additionally, metabolic processes produce acid and alkali. Metabolic processes that convert cationic compounds to neutral products generate hydrogen ions, whereas those that convert anionic compounds to neutral products consume hydrogen ions (DiBartola, 2012; Koeppen and Stanton, 2013). The primary sources of acid are the oxidation of the sulfur-containing amino acids such as methionine and cysteine, cationic amino acids, such as lysine and arginine and hydrolysis of organic phosphate di-esters, such as phospholipids and nucleic acids (Allen, 1996; Markwell, Buffington and Smith, 1998; DiBartola, 2012). A short amount of base is lost each day from the gastrointestinal tract (primarily as organic anions), and this is equivalent to a gain of fixed acid (Koeppen, 2009; DiBartola, 2012). The main sources of bases are the metabolism of anionic amino acids such

as glutamate and aspartate, the oxidation of other organic anions such as lactate and citrate, or their use for gluconeogenesis (DiBartola, 2012; Koeppen and Stanton, 2013).

That persisting discrepancy between acid and base production determines the net endogenous acid production rate, the net acid load of the diet, which consecutively determines the level of perturbation of systemic acid-base equilibrium (Frassetto *et al.*, 1998; Oh, 2000).

In order to estimate diet net acid load, measurements of urinary excretion of ammonium, titratable acids and bicarbonate, designated net acid excretion - NAE, are required, or it can be calculated from dietary constituents, designated net endogenous acid production - NEAP (Frassetto *et al.*, 2007; Pizzorno, Frassetto and Katzinger, 2010). Food provides a net acid or base effect as a result of the balance between the acid-forming constituents and the base forming constituents (Koeppen, 2009; Pizzorno, Frassetto and Katzinger, 2010).

The gastrointestinal absorption of nutrients and other dietary components is very important in determining acid production. The impact of an acid or an alkali on the body's acid-base balance depends essentially on their absorption. Overall, the amount absorbed is approximately equal to the amount ingested, if the substance is soluble and is easily absorbed like, for instance, in the case of potassium citrate and ammonium chloride. An insoluble substance such as calcium carbonate must react with gastric acid or acid in food to become soluble and absorbable. The effects of these substances on the body's acid-base balance are complex and are frequently unrelated to the acidity or alkalinity of the substance, but on the interaction with other chemicals, both endogenous and exogenous (Oh, 2000).

Moreover, the liver oxidizes the absorbed sulfur-containing amino acids and several organic anions (Remer, 2000). When organic anions are metabolized to carbon dioxide and water, bicarbonate is produced, and the opposite occurs when the absorbed sulfur-containing amino acids are metabolized (Markwell, Buffington and Smith, 1998). Before being released from the corresponding cells into the circulation, these ions are primarily buffered by intracellular fluid buffers. Combining the acid-base pool originated from the diet they are going to be buffered by extracellular fluid buffers in conjunction with pulmonary mechanisms and later excreted by the kidneys (Remer, 2000).

Thus, these four different organs, intestine, liver, lungs, and kidneys play an important role in the acid-base metabolism (**Figure 2**).

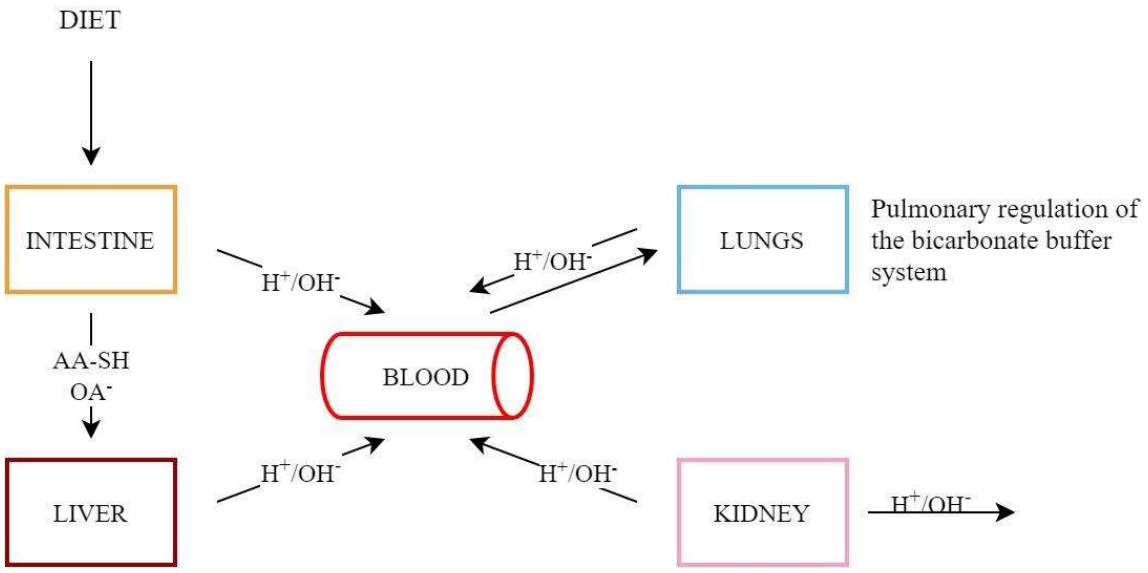


Figure 2: Interaction of organs in acid-base metabolism. H^+: hydrogen ions; OH^-: primarily HCO_3^-; AA-SH: sulfur-containing amino acids; OA: alkali salts of non-metabolizable organic acids. Every day, acid and alkali are ingested in the diet. The GI system absorbs acid and alkali, which are then metabolised in the liver, and posteriorly buffered by extracellular fluid buffers in conjunction with pulmonary mechanisms and later excreted by the kidneys. Adapted from: (Remer, 2000).

2. Urinary pH

Urinary pH is a rough but helpful estimate of acid-base balance, however, is not a reliable indicator of blood pH since it is highly influenced by diet and recent feeding. In dogs and cats, the normal values may fluctuate between 5.0 to 8.5 (Barsanti, 2012). Urinary pH is a parameter which varies between individuals, with some variations between breeds (Stevenson and Markwell, 2001). Within the same individual, there is an inverse association between urinary pH and age, and body condition score (BCS) may also play a role (Kennedy *et al.*, 2016). In human medicine, there is a strong inverse relationship between urinary pH and body weight in subjects with nephrolithiasis (Maalouf *et al.*, 2004; Najeeb *et al.*, 2013). Still, to the authors' knowledge to the date, no study demonstrated the same association in dogs.

Many additional factors influence urinary pH. Acidic urine can be due to ingestion of high protein diets, respiratory and metabolic acidosis, severe vomiting with chloride depletion, severe diarrhoea, Fanconi syndrome, diabetes mellitus in particular if the animal is in ketoacidosis, starvation, pyrexia, and administration of urinary acidifiers (Chew and DiBartola, 1998; Barsanti, 2012; DeNicola *et al.*, 2017). Causes of alkaline urine include renal tubular acidosis (RTA), ingestion of alkali, diets rich in vegetables and cereals, a recent meal (postprandial alkaline tide), urinary tract infections (UTI) with urease-producing bacteria (typically Staphylococcus or Proteus *spp.*) and metabolic and respiratory alkalosis (Chew and DiBartola, 1998; Stevenson and Markwell, 2001; Barsanti, 2012). If the urine unit container is

allowed to stand open to air at room temperature (which leads to loss of CO_2) or to be contaminated by detergents or disinfectants, urinary pH may increase unintentionally (Chew and DiBartola, 1998; Albasan *et al.*, 2003).

3. Urinary pH measurement - pH meter *vs.* pH strips

Urinary pH is a valuable and easily measurable biochemical marker and a vital part of the complete urinalysis as it is essential for the interpretation of urine chemistry and sediment findings and may reflect systemic acid-base abnormalities. In patients with uroliths, it can be used to determine the need for urinary pH manipulation and help monitor responses to treatment. There are many ways that urinary pH may be measured, although the two most common are the dipstick testing and the use of a pH meter (Lanevschi-Pietersma, 2002; Barsanti, 2012; Kwong *et al.*, 2013; Athanasiou *et al.*, 2018).

A calibrated pH meter is regarded as the gold standard. However, it is much less regularly operated than dipstick testing in veterinary medicine (Athanasiou *et al.*, 2018). What makes his use much less frequently is that pH meters are more time-consuming as periodic calibration with test solutions and user training is needed (Kwong *et al.*, 2013). There are two types of instruments: portable pH meters and benchtop pH meters. Benchtop pH meters include electrodes that enable hydrogen ions in a solution to move through a selective barrier, evoking a measurable voltage proportional to the solution's pH (Johnson, Lulich and Osborne, 2007).

In contrast, dipsticks are single-use test strips that can measure a range of variables in addition to pH, including the presence of glucose, protein, leucocytes, and nitrite. They require much less user training, are cheaper to use, can be used in clinical practice and have the possibility to be used outside the clinic. Strip reagent pads are impregnated with different dyes that change color based on pH (Athanasiou *et al.*, 2018). The intensity of the color change is proportional to the concentration of the substance being measured (Lanevschi-Pietersma, 2002). Dipstick measurement of the leukocytes, USG (urine specific gravity), creatinine and UPC (Urine Protein:Creatinine) ratio, however, are not recommended for canine and feline urine (Holan *et al.*, 1997; Bauer, Rettig and Moritz, 2008), although one study claims that dipsticks can be used in a clinical setting as a highly specific test for estimation of the UPC ratio in dogs and cats (Defontis *et al.*, 2013). Another problem with strips is that it can lead to variations in assessment between individuals since color variations may be subtle, and individual visual acuity for color perception can vary (Lanevschi-Pietersma, 2002; Raskin, Murray and Levy, 2002).

One study comparing methods of urinary pH measurement in dogs showed that dipstick measurement of pH resulted in an overestimation of the pH value (Johnson, Lulich and Osborne, 2007). Several instruments are commercially available for reading reagent test strips. In human literature, with the appearance of electronic readers, readings are less predisposed to perception bias in dipstick urinalysis (Tighe, 1999; Rumley, 2000). The likewise was also registered in companion animals, as automated reading of the dipsticks was better than visual reading and the better method for urine dipstick examination (Defontis *et al.*, 2013; Ferreira *et al.*, 2018). This technology is based on the principle of reflectance; the amount of light reflected is inversely proportional to the concentration of the substance present (Lanevschi-Pietersma, 2002). However, since these dipsticks were initially designed for humans, some of the test pads are not useful or reliable in animal species, and consequently, validation of dipsticks has to be performed before use in veterinary species (Bauer, Rettig and Moritz, 2008).

Considering clinically relevant disparities occur with an unacceptable frequency, it is suggested that the dipsticks may only be applied when obtaining pH approximations for routine urinalysis but are not recommended when regular and accurate pH measurements are critical for diagnosis, prevention, and management of a disease. Urinary pH should be determined by a pH meter in these scenarios (Chew and DiBartola, 1998; Defontis *et al.*, 2013; Kwong *et al.*, 2013; Athanasiou *et al.*, 2018).

4. Urinary pH estimation

Food and endogenous metabolic processes are the sources of acid or base intake or production. Biochemical analyses of food confirm that almost all foods contain acid precursors, while fruits and vegetables also contain base precursors. Meat intake has an acidifying effect due to its high content of sulfuric amino acids (methionine and cysteine), fruits and vegetables provide mainly an alkali load due to the extensive amounts of potassium and organic anions it contains (Skoch *et al.*, 1991; Frassetto *et al.*, 2018). Based on this information, dietary formulas for estimating the acid or base effects of different foods have been developed. Application of estimates of dietary intake bypasses having to measure renal net acid excretion. Nonetheless, these formulas require quantitative analyses of both dietary cations (sodium, potassium, calcium, magnesium) and anions (chloride, sulfate, phosphate).

a. Cations and anions

Electrolytes can be either cations or anions. The primary extracellular ions are sodium, chloride, potassium, calcium, phosphate, and magnesium ions (Guyton and Hall, 2011; VanPutte, 2016). They can be found in the food and water ingested and removed from the body by the kidneys and, to a lesser degree, the liver, skin, and lungs (VanPutte, 2016).

i. Sodium

Sodium ions are the predominant extracellular cations (VanPutte, 2016). The primary route by which Na^+ is excreted is throughout the kidneys. Sodium ions promptly crosses from the glomerulus inside the lumen of the Bowman capsule. The amount of Na^+ and water reabsorbed in the renal tubule delimits the concentration of Na^+ excreted in the urine. Large quantities of Na^+ are excreted in the urine when the reabsorption from the tubule decreases, and on the contrary, only small quantities are excreted in the urine when tubule reabsorption increases (Koeppen and Stanton, 2013; VanPutte, 2016). Although in the proximal convoluted tubule the Na^+ transport rate is moderately consistent, in the distal convoluted tubule and the collecting duct, the mechanisms accountable for the Na^+ transport are under hormonal control (Guyton and Hall, 2011; VanPutte, 2016).

Studies of healthy adult humans (Hughes and Norman, 1992; Curhan *et al.*, 1997) and dogs (Lekcharoensuk *et al.*, 2002; Allen *et al.*, 2015) indicate that high dietary sodium consumption generates hypercalciuria. Because of that, high sodium intake is currently not recommended in human stone formers. Effects of sodium chloride supplementation have been studied in companion animals with variable outcomes (Lulich *et al.*, 1999, 2004; Lulich, Osborne and Sanderson, 2005; Queau *et al.*, 2020). In a few studies urinary concentrations of calcium were not significantly affected by dietary sodium concentration even though calcium excretion increased. A probable explanation is that although increased dietary sodium intake does increase urinary calcium excretion, it increases urine volume, which may decrease urine calcium concentration (Lulich *et al.*, 2004; Lulich, Osborne and Sanderson, 2005; Queau *et al.*, 2020). High sodium diets result in higher urinary sodium concentrations since this nutrient is easily absorbed in the gastrointestinal tract and predominantly eliminated by the kidneys, which regulate the glomerular filtration and tubular reabsorption to keep homeostasis (Queau *et al.*, 2020).

ii. Chloride

The predominant anions in the extracellular fluid are chloride ions (Autran de Morais and Biondo, 2012; VanPutte, 2016). Chloride is essential for maintaining osmolality, and solidly engages in acid-base regulation. The kidneys perform an essential part in controlling plasma chloride concentration. After sodium, chloride is the most common ion in the glomerular ultra-filtrate. The filtered chloride is almost entirely reabsorbed in the renal tubules (Autran de Morais and Biondo, 2012). The mechanisms that regulate sodium, potassium and calcium levels in the body are essential in determining chloride levels. The mechanisms that regulate sodium extracellular concentration are the most influential for regulating extracellular chloride concentration since Na^+ is the predominant cation (Guyton and Hall, 2011; VanPutte, 2016).

iii. Potassium

Potassium ion concentration has to be very thoroughly controlled across the plasma membrane because numerous cell functions are susceptible to shifts in extracellular fluid potassium concentration. For example, an expansion in plasma potassium concentration generate cardiac arrhythmias and can lead to cardiac arrest or fibrillation (Guyton and Hall, 2011; VanPutte, 2016). The majority of the total body potassium is contained inside the cells. If ingested potassium does not rapidly move into the cells, after ingestion of a typical meal, the extracellular fluid potassium concentration would increase to a harmful level. Both insulin and aldosterone increase cell potassium uptake (Guyton and Hall, 2011). The amount of potassium excreted in the feces is only a tiny per cent of the potassium intake, maintenance of balance between intake and output of potassium depends primarily on the kidneys' excretion (Guyton and Hall, 2011; VanPutte *et al.*, 2016). Potassium ions are promptly filtered out of the blood in the renal corpuscle. The proximal convoluted tubules and loop of Henle fully reabsorb potassium ions and the distal convoluted tubules and collecting ducts secret them. Potassium ion secretion into the distal convoluted tubules and collecting ducts is highly regulated and primarily responsible for controlling the extracellular concentration of K^+ (VanPutte, 2016). It was observed that reduced dietary potassium intake increased urinary calcium excretion and that potassium supplementation reduced calcium excretion (Lekcharoensuk *et al.*, 2002).

iv. Calcium

Similarly to other ions, the extracellular concentration of calcium ions is controlled within a narrow range. The kidneys, digestive tract, and bones are critical in maintaining extracellular Ca^{2+} levels. Unlike ions before-mentioned as sodium and chloride, a large portion of calcium excretion happens in the feces (Guyton and Hall, 2011; VanPutte, 2016). Dietary calcium absorption from the intestines is held with the aid of vitamin D and parathyroid hormone (PTH) with any excess being excreted in the urine. There is a strong relationship between urinary calcium excretion and dietary calcium intake in humans, except for individuals who are hyper absorbers of calcium (Stevenson *et al.*, 2004). There are indications that dogs forming calcium oxalate stones might also be hyper absorbers of calcium (Lulich *et al.*, 1999; Stevenson *et al.*, 2004). Calcium is absorbed in the ionic state, and consequently, substances such as phosphate, citrate, sulphate, oxalate, and fatty acids that complex with calcium either in the diet, or gut, diminish the availability of calcium for absorption (Lulich *et al.*, 1991; Stevenson, Hynds and Markwell, 2003; Stevenson *et al.*, 2004). Most of the calcium in the body is deposited in the bone. The bone, consequently, functions as a great storage system and as a source of calcium when there is a drop in extracellular fluid calcium concentration. PTH is essential in managing bone uptake and release of calcium (Guyton and Hall, 2011).

This hormone secreted by the parathyroid glands augments the extracellular levels of Ca^{2+}; on the other hand, it decreases the extracellular levels of phosphate. Besides extracellular Ca^{2+} levels regulate the rate of PTH secretion, when they are increased, PTH secretion is inhibited, and when they are decreased is stimulated. The osteoclastic activity is increased by the PTH, which in turn culminates in bone degradation that causes the release of Ca^{2+} and phosphate ions to the blood. The Ca^{2+} reabsorption from renal tubules and the phosphate ions urine concentration are both increased by PTH, and it furthermore enhances the rate at which vitamin D is converted to active vitamin D. This active vitamin D is vital because it increases Ca^{2+} absorption over the intestinal mucosa. Accordingly, the regulative agents that control intestinal calcium absorption and secretion and the gastrointestinal tract perform a vital part in calcium homeostasis (Guyton and Hall, 2011; VanPutte, 2016). Another factor that affects calcium reabsorption is the plasma concentration of phosphate (Riond, 2001).

In the kidneys, calcium is both filtered and reabsorbed but not secreted. Regularly, the majority of the filtered calcium is reabsorbed by the tubules, with only a scarce percent of the filtered calcium being excreted (Guyton and Hall, 2011; VanPutte, 2016). Increased plasma phosphate reduces calcium excretion by stimulating PTH, which enhances calcium reabsorption by the renal tubules. In contrast, diets deficient in phosphorus may stimulate calcitriol production,

which, in turn, increases intestinal absorption of both calcium and phosphorus (Lulich *et al.*, 1999; Lekcharoensuk *et al.*, 2002; Stevenson *et al.*, 2004). The hypocalciuric influence of dietary phosphorus is strongly supported in humans (Spencer *et al.*, 1978).

v. Phosphorus

Phosphorus is a mineral present in many mammals. It is crucial for bone mineralization and executes an essential role in indispensable processes like energy metabolism (Guyton and Hall, 2011; Alexander and Britta, 2018).. The vast majority of phosphate in the body is in the form of calcium phosphate salts in the bone. The most substantial part of the remaining phosphate is inside cells. Many of the phosphate ions are covalently bound to other organic molecules such as lipids (to form phospholipids), proteins, and carbohydrates. Phosphates also play essential roles in regulating enzyme activity and can act as buffers as discussed previously. Phosphate ions can be presented in multiple forms such as $H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-} with the most common being HPO_4^{2-}. The ability of the kidneys to reabsorb phosphate ions is restricted (Guyton and Hall, 2011; VanPutte, 2016). The kidneys excrete phosphate fundamentally by an overflow mechanism. The renal tubules hold an average transport maximum for reabsorbing phosphate. When that amount of phosphate present in the glomerular filtrate does not reach the maximum, virtually all the filtered phosphate is reabsorbed. If it does, the excess is excreted (Guyton and Hall, 2011). Over time, a diet low in phosphate can increase the re-absorptive transport maximum for phosphate, thereby reducing the tendency for phosphate to be excreted in the urine. Consequently, most of the filtrated phosphate is reabsorbed to preserve the extracellular phosphate concentration (Lulich *et al.*, 1999; Lekcharoensuk *et al.*, 2002; Stevenson *et al.*, 2004). PTH serves as an essential element on the extracellular phosphate levels regulation by promoting bone reabsorption, which delivers Ca^{2+} and phosphate ions into the ECF, and by decreasing the transport maximum for phosphate by the renal tubules, so a more significant proportion of the tubular phosphate is lost in the urine. Ca^{2+} and phosphate ions are going to precipitate as calcium phosphate salts in soft tissues if phosphate levels in the extracellular fluid rise above average (VanPutte, 2016). As an outcome of a critically reduced rate of filtrate formation by the kidneys in cases of acute or chronic renal failure, high phosphate blood levels may be noticed. Moreover, it has been reported that dietary P restriction prevents not only secondary hyperparathyroidism but also prevents parathyroid cell growth (Slatopolsky *et al.*, 1996).

vi. Magnesium

Like phosphate, most of the magnesium in the body is stored in the bones or intracellular fluid (ICF) with only a small per cent located in the ECF. More than one half of this is bound to plasma proteins, and the rest is free. Magnesium is involved in multiple biochemical processes in the body, including activation of several enzymes, such as the sodium-potassium pump involved in actively transporting Na^+ out of and K^+ into cells (Guyton and Hall, 2011; VanPutte, 2016). Low and high levels of plasma magnesium exhibit symptoms associated with the effect of magnesium on Na^+–K^+ active transport. Additionally, excessive magnesium intake seems to increase urinary calcium excretion in dogs (Lekcharoensuk *et al.*, 2002) and humans (Fetner *et al.*, 1978). The gastrointestinal tract absorbs only about one half of magnesium intake. In order to maintain magnesium balance, the kidneys must excrete this absorbed magnesium. Free Mg^{2+} moves into the filtration membranes of the kidney into the filtrate. The loop of Henle holds the primary site of reabsorption with the majority of ions being reabsorbed from the filtrate. The proximal convoluted tubule, distal convoluted tubule, and collecting duct reabsorb the rest (VanPutte, 2016). The mechanisms that control Mg^{2+} reabsorption are not entirely clear, but increased extracellular fluid magnesium concentration, extracellular volume expansion, and increased extracellular fluid calcium concentration lead to increased magnesium excretion (Guyton and Hall, 2011).

Information is scarce regarding the metabolism of some minerals in metabolic acidosis, but phosphorus, magnesium, potassium, chloride and sodium metabolism may be variably affected. Metabolic acidosis due to a reduction in filtration and reabsorption in the proximal renal tubule of bicarbonate may affect sodium and chloride renal handling (Ching *et al.*, 1989). Chloride excretion plays an essential role in the adaptation of the kidneys to metabolic acidosis and chronic respiratory acid-base disturbances. The kidneys increment net acid excretion primarily in the form of NH_4Cl (Autran de Morais and Biondo, 2012; VanPutte. *et al.*, 2016).
Acid-Base abnormalities can also affect potassium distribution. Metabolic acidosis induces loss of potassium from the cells, increasing extracellular potassium concentration, while metabolic alkalosis reduces extracellular fluid potassium concentration (Guyton and Hall, 2011; VanPutte, 2016). Bone is involved as a buffering system for acid-base control of body fluids (Ching *et al.*, 1989; Guyton and Hall, 2011; VanPutte, 2016).

Through metabolic acidosis, induced by, per example, anionic supplements, phosphates and carbonates mobilized from the bone to buffer the metabolic acidosis are going to offset the acidifying metabolites (Stevenson and Markwell, 2001; Stratton-Phelps and House, 2004). Bone calcium is released with the phosphorus resulting in hypercalciuria. During metabolic acidosis, the activity of osteoclasts and osteoblasts are increased and decreased, respectively, stimulating the physicochemical mineral and the cell-mediated bone resorption dissolution. Bone density may be affected by prolonged metabolic acidosis (Oh, 2000; Riond, 2001; Stevenson and Markwell, 2001).

Calcium reabsorption is also stimulated by metabolic acidosis and inhibited by metabolic alkalosis. Most of the effect of hydrogen ion concentration on calcium excretion results from changes in calcium reabsorption in the distal tubule (Guyton and Hall, 2011; VanPutte, 2016). Metabolism of magnesium is comparable to that of calcium, in acidosis (Ching *et al.*, 1989). Along with redistribution among intracellular and extracellular compartments, there is also urinary magnesium increased excretion and mobilization (Ching *et al.*, 1989; Guyton and Hall, 2011; VanPutte, 2016).

b. Base Excess

The ingestion of macro-elements alters the metabolism of animals, resulting in the metabolic adaptation of the buffer systems. Urinary pH is significantly influenced by feeding, whereby the influence that food has on the pH of the urine depends on its ingredients, not on the pH of the food itself (Izquierdo and Czarnecki-Maulden, 1991; Jeremias *et al.*, 2013). An alternative approach to acid-base balance was proposed in the early 1980s by Peter Stewart. Rather than concentrating solely on the equilibrium of carbonic acid, as traditional methods do, this approach demands a distinction between independent and dependent variables involved in acid-base balance. He considered that variables commonly used for acid-base estimation like pH or bicarbonate are dependent variables and the independent variables, to be the strong ion difference, the partially dissociated weak acids (albumin, phosphate), and the partial pressure of carbon dioxide (PCO_2) of the solution (Riond, 2001; Story, 2004). The term strong ions refer to the deeply dissociated non-metabolizable ions. Hence, the difference between the total number of strong cations and anions in the blood is called the strong ion difference. The principal cations present in food are Na^+, K^+, Ca^{2+}, and Mg^{2+}. The principal anions found in food are Cl^-, SO_4^{2-}, and $H_2PO_4^-$ (Riond, 2001; Jeremias *et al.*, 2013). The strong ion difference

of the blood can only be altered if the cations/anions are absorbed into the blood. The pH is determined based on the difference between the number of cations and anions absorbed from the diet. The addition of anions to food produces metabolic acidosis from a compensatory rise in extracellular hydrogen ions. The excess H^+ is excreted by the kidneys to preserve electro-neutrality, producing urine of a lower pH (Riond, 2001; Jones, Streeter and Goad, 2009; Pizzorno, Frassetto and Katzinger, 2010). A few studies have proven the influence of food cation and anion balance on the intermediary metabolism of dogs and cats (Ching *et al.*, 1989; Kienzle, Schuknecht and Meyer, 1991; Kienzle and Wilms-Eilers, 1994; Wagner, Keusch and Iben, 2006; Yamka and Mickelsen, 2006; Autran de Morais and Constable, 2012; Jeremias *et al.*, 2013).

Estimation of food base excess (BE) from the concentrations of alkaline and acid compounds in the food has been assessed as a practical method for predicting the influence of food on urinary pH. The ratio of cations to anions can be estimated applying a formula in which the molar proportions and the valence of the ions are taken into account. Various formulas have been formulated over the years by different authors (Kienzle, Schuknecht and Meyer, 1991; Kienzle and Wilms-Eilers, 1994; Markwell, Buffington and Smith, 1998; Yamka and Mickelsen, 2006). Food that presents a relative predominance of cations has a positive food BE and promotes alkaline urine formation, whereas the opposite occurs in foods with a relative predominance of anions. Kienzle, Schuknecht and Meyer (1991) research was undertaken to determine a suitable method to estimate the urinary pH in cats through analyzing their diet. It was confirmed that under practical conditions, the influence of a diet on urinary pH could be estimated of the base excess in the food. Kienzle and Wilms-Eilers (1994), in the second part of the investigation, assessed the effect of a basal diet with different amounts or combinations of acidifying and alkalizing additives fed to six healthy cats. The base excess in the food (mmol/kg dry matter) was calculated (unlike the first part of the investigation, in this investigation minerals and amino acids were given in g/kg DM instead of mmol, and the factors included valence and molecular weight) as follows:

BE = (49.9 × calcium) + (82.3 × magnesium) + (43.5 × sodium) + (25.6 × potassium) - (64.6 × phosphorus) - (13.4 × methionine) - (16.6 × cystine) - (28.2 × chloride)

In one study performed with dogs, it was not possible to determine any influence on the pH value by the calcium or magnesium content of the rations used. However, it was observed a

relationship between the substances ingested and the urinary pH of dogs. There was a direct correlation between an increase in potassium and sodium in the food and an increase in the urinary pH value, on the contrary, higher amounts of sulfur, phosphorus or chloride in the food produced a drop in the pH value. The following equations for the estimation of urinary pH were established (quantity elements are to be used in g/100g DM) (Yamka and Mickelsen, 2006):

Urinary pH (wet and dry foods) = 7.30 + (0.54 × sodium) + (0.63 × potassium) - (0.53 × chloride) - (1.67 × sulfur) - (0.61 × phosphorus) + (2.07 × cystine)

Urinary pH (wet foods) = 6.97 + (1.37 × sodium) + (1.24 × potassium) - (0.98 × chloride) - (3.19 × sulfur) - (0.58 × phosphorus) + (1.06 × methionine) + (1.03 × cystine)

Urinary pH (dry foods) = 8.09 - (1.15 × sulfur) - (0.50 × phosphorus) - (0.16 × methionine)

Yamka and Mickelsen (2006) measured the urinary pH twice per day. It was not performed a collection of all the urine produced in 24 hours. Circadian variations in urinary pH are significant, particularly throughout the postprandial period. Therefore, interpreting the pH value of only one or two urine samples, especially when the feeding time is not considered, might not hold the most accurate method for determining overall urinary pH. Despite existing a fair number of studies that support the idea of determining urinary pH through the estimation of the food BE, only a recent study has been performed in dogs and accounts for some controversy regarding the accuracy and validity of these estimations (Yamka and Mickelsen, 2006). Urinary pH prediction from the dietary analysis can be a valuable tool. However, it has to be kept in mind that there are a number of sources of inaccuracies and variations involved. Significant additional research, particularly in dogs, is required.

5. Postprandial alkaline tide effect

The alkaline tide effect is the designation given to the physiological process regarding the alkalization of the blood and urine, *i.e.* rise in the pH, following a meal. This increase can be characterized by stimulation of the hydrochloric acid secretion in the stomach generated over the food intake. If insufficient acidifying ions are absorbed, this drives to a loss of H^+ ions from the body fluids. The kidney counteracts the resulting metabolic alkalosis with increased

excretion of alkaline ions (HCO_3^-) through the urine, the urine pH will consequently rise (Rune, 1965, 1966; Brooks, 1985; Niv and Fraser, 2002). Urinary pH is not constant and may fluctuate over the 24 hours. Due to these diurnal changes, urinary pH is regularly raised in the daytime and lower in the early morning and at night (Elliot, Sharp and Lewis, 1959).

Diurnal variation in urinary pH was reported in humans (Cameron *et al.*, 2012). The following studies also demonstrated this event in dogs (Ozaki *et al.*, 2000; Stevenson *et al.*, 2000; Middelbos *et al.*, 2006) and cats (Finke and Litzenberger, 1992), a postprandial alkaline tide occurred between 1 and 5 hours after the morning meal, although this finding was not always uniformly observed (Elliot, Sharp and Lewis, 1959; Gleaton, Bartges and Laflamme, 2001). Gastric acid secretion with the concomitant alkalization of plasma has been stated to be the origin of postprandial changes in plasma pH and urinary pH (Cameron *et al.*, 2012). Some even have studied the applicability of the alkaline tide as a clinical measure of acid secretion (Rune, 1965; Forster *et al.*, 1972). The administration of a proton pump inhibitor would inhibit the alkaline tide if gastrointestinal acid or base secretion were responsible for this diurnal pH variation. In some studies, it was discovered that surgical vagotomy/vagal denervation (Brooks, 1985; Johnson and Rai, 1990; Johnson, Harris and Wastell, 1990), anti-secretory agents (Johnson and Rai, 1990; Ozaki *et al.*, 2000) and some acidifiers (Kienzle and Wilms-Eilers, 1994) led to the suppression of the alkaline tide. Nevertheless, there is conflicting evidence since other studies noted a postprandial alkaline tide notwithstanding inhibition of gastric acid secretion (Vaziri *et al.*, 1980; Johnson, Mole and Pestridge, 1995; Cameron *et al.*, 2012).

It has been proposed that the extent of the postprandial alkaline tide is a reflection of the amount of food consumed. Finke (1992) found postprandial urinary pH to be conditioned by food intake and it could be represented by a simple linear model, it also suggested that urinary pH would plateau at a level, reflecting the acid-base characteristic of the diet fed, instead of continuing to rise with increasing food intake.

Considering that the duration of the buffering capacity from protein in the stomach depends upon the velocity of emptying, effects on gastric emptying must also be thought out. Feeding *ad libitum* can be valuable in reducing the extent of the alkaline tide, and this may be especially helpful in prevention of struvite formation (Brooks, 1985; Finke and Litzenberger, 1992).

There is indication of a physiologic acid-base rhythm with a curve of diurnal fluctuation which is characteristic for a given individual and it is a result of several factors such as diet, emotional status, exercise, personality and pulmonary ventilation (Elliot, Sharp and Lewis, 1959). Nevertheless, the specific circadian profile of urine acidification remains imperfectly defined.

In order to formulate recommendations concerning feeding management, it is essential to know the effect of food intake on postprandial urinary pH.

6. Urinary pH manipulation

As discussed earlier, nutrition may thoroughly influence acid-base balance in animals. Diets can be formulated to produce acidic or alkaline urine. The acidification potential of a diet depends on its ingredients and the balance between acidifiers like methionine, calcium or sodium sulfate, ammonium chloride or alkalizers like calcium carbonate and potassium citrate (Finke and Litzenberger, 1992; Queau, 2019). Diet manipulation is reported to be a tool to prevent the hypocalcemic postparturient paresis of dairy cows (Riond, 2001). Dietary alkali is also believed to improve the continues decline in glomerular filtration rate possibly caused by acid retention (Wesson and Simoni, 2009; Scialla, 2015; Goraya *et al.*, 2019). Another important practical application is by enhancing the renal elimination of certain toxins/drugs (Grunberg, Morse and Barrett, 1983; Plumb, 2005). In humans, the oral administration of acid is generally used to accelerate the elimination of phencyclidine, amphetamine and methamphetamine, since basic drugs are quickly eliminated in acid urine (Beckett, Rowland and Turner, 1965; Simpson and Khajawall, 1983; Wang *et al.*, 2009). While beneficial, it must be used with caution as over-acidification has been shown to cause a wide variety of problems, including calcium and potassium depletion. A low degree of acidosis can become meaningful if the acidosis is present for more extended periods (Mousa, 2016).

Except for specific prescription diets designed to alter a dog's urinary pH level to prevent the formation of bladder and kidney stones, most commercially prepared dog foods tend to have acidic pH levels. The benefits of alkaline diet as well as alkaline water have been described in humans, and may also be advantageous in dogs and cats (Abol-enein *et al.*, 2009; Frassetto *et al.*, 2018). Consumption of abundant alkaline-forming foods can result in improvement in bone mineral density, protection from chronic illnesses, reduced tumor-cell invasion and metastasis, and effective excretion of toxins from the body (Mousa, 2016; Frassetto *et al.*, 2018). In addition, a large number of studies showing the benefits of alkaline water have shown a lower incidence of cardiovascular disease, cancer and lower total mortality rates (Mousa, 2016).

Urinary pH manipulation is essential in some types of urolith formation, considering urine acidity or alkalinity influences what type of mineral precipitates (Gleaton, Bartges and Laflamme, 2001). Cystine solubility can be enhanced by inducing an alkaline urinary pH, and

uric acid stones are easily formed in acidic urine (Murayama and Taguchi, 1993). Struvite is less soluble and therefore more likely to precipitate in alkaline urine, consumption of a diet formulated to dissolve struvite uroliths containing a urinary acidifier is shown to promote struvite urolith dissolution in dogs and cats when compared with maintenance adult foods (Abdullahi *et al.*, 1984; Murayama and Taguchi, 1993; Funaba *et al.*, 2003; Tion, Dvorska and Saganuwan, 2015; Lulich *et al.*, 2016; Torres-Henderson *et al.*, 2017). Calcium oxalate stones tend to develop in acidic urine. However, the effect of urinary pH on the risk of calcium oxalate remains controversial (Lulich *et al.*, 1999; Stevenson *et al.*, 2000; Funaba *et al.*, 2003; Chutipongtanate, Chaiyarit and Thongboonkerd, 2012; Kennedy *et al.*, 2016).

Food can be a valuable tool in altering pH, establishing pH profiles for multiple ingredients will serve in the development of diets designed to control urinary pH, without non-nutritional acidifiers.

a. Acidifiers

Sulfur is known to have a strong influence on the acid-base status and may be decisive to regulate urinary pH. Sulfur can be added to diets through sulfur-containing amino acids, like methionine and cysteine (Halfen *et al.*, 2018). Methionine and cysteine are frequently considered together being methionine a precursor to cysteine (Rumbeiha and Morrison, 2011).

In pigs, the inclusion of **calcium sulfate** ($CaSO_4$) to the diet, also led to an acidic urinary pH (Canh *et al.*, 1998). However, in dogs, the addition of calcium sulfate with a sulphur amount of either 8 or 15 g/kg DM was followed by a small, but not significant, urinary acidification (Janczikowski, Wolf and Kamphues, 2008). Results of another study on cats indicate that the administration of calcium sulfate at two levels, 1.28 and 2.56g S/kg of diet, into a basal extruded dry cat food with base excess (BE) around 100mEq/kg, did affect mean urinary pH (Halfen *et al.*, 2018).

This may be explained by the alkalizing effect of calcium, which nonetheless was not strong enough to withdraw the acidifying effects of sulfur. Furthermore, Ca^{2+} has low urinary excretion and blood circulation (Guyton and Hall, 2011).

Distinct formulations of **methionine** have been applied in animals to treat diseases and give nutritional supplementation (Hickey, Son and Wismer, 2015). DL-methionine (DLM) can be used to correct the dietary amino acid balance and also as a urinary acidifier (Funaba *et al.*, 2001; Halfen *et al.*, 2018). Unlike humans, dogs and cats appear to utilize the D- and L-isomer

of methionine equally efficiently (Cho *et al.*, 1980; Burns and Milner, 1981; Funaba *et al.*, 2001). Multiple studies verify the effectiveness of L-methionine/DL-methionine as a urine acidifier in both humans, cats, and dogs (Funaba *et al.*, 2001; Hickey, Son and Wismer, 2015; Siener, Struwe and Hesse, 2016; Halfen *et al.*, 2018). Though in one study conducted in dogs, urinary pH was not influenced by 0.1 or 0.2% of supplemental methionine concentration, both pre-prandial and postprandial. Still, the study design concerning urine collections was not very accurate (Middelbos *et al.*, 2006).

The benefit of pharmacotherapy with methionine in contrast to meat or fish is that the intake of other dietary elements such as dietary calcium, phosphate, sodium, and purines is avoided. Direct markers for the metabolism of methionine, urinary sulfate (metabolic waste product) excretion, and ammonium excretion (reflects the increase in net acid production), are significantly increased after methionine administration (Siener, Struwe and Hesse, 2016).

Methionine is quickly and efficiently absorbed in the small intestine and metabolized in the liver, generating sulfate ions and protons (Siener, Struwe and Hesse, 2016). While beneficial, methionine it is also considered the most toxic amino acid in food supplements due to its small dosing spectrum and adverse effects (Finke and Litzenberger, 1992; Hickey, Son and Wismer, 2015). The consequences of long-term excessive methionine ingestion or acute methionine toxicity have been described in dogs, cats, and people (Fau *et al.*, 1987; Garlick, 2006; Rumbeiha and Morrison, 2011; Hickey, Son and Wismer, 2015). Methionine is metabolized in the liver by two distinct pathways, the transamination pathway and the transsulfuration-transmethylation pathway (Hickey, Son and Wismer, 2015).

Two metabolites of methionine seem to cause the bulk of toxic effects: homocysteine and methanethiol. Methionine intoxication in dogs can affect both the GI tract and the nervous system (Hickey, Son and Wismer, 2015). Six hunting dogs poisoned by methionine presented ataxia, disorientation, hyperactivity, lethargy, ptyalism, tremors, and vomiting. Following consumer complaints, batches of a dog's food were analyzed, and the concentration of methionine was discovered to be in the range of 1.60–2.75% (Rumbeiha and Morrison, 2011). Furthermore, records of dogs with presumptive methionine ingestion were reviewed from the American Society for the Prevention of Cruelty to Animals Animal Poison Control Center database, with ingested methionine doses ranging from 3.9 to 23.462 mg/kg BW. Nevertheless, with decontamination and supportive care, signs usually resolve within 48 hours (Hickey, Son and Wismer, 2015).

Excessive amounts of methionine (1 g/kg BW/day) produce methemoglobinemia and Heinz body formation in erythrocytes, resulting in hemolytic anemia in cats (Maede *et al.*, 1987). Methionine should not be given to animals with preexisting acidosis or with oxalate or urate calculi. It is contraindicated in patients with renal failure or pancreatic disease and is not recommended for treatment in kittens (Plumb, 2005). FEDIAF recommends 0.88g of methionine for early growth and reproduction, 0.65g for late growth and 1.00g for adult maintenance per 1000 kcal of ME for the dog and 1.10g methionine for growth and reproduction with a maximum of 3.25g for growth and 0.43g for adult maintenance per 1000 kcal of ME for the cat (The European Pet Food Industry, 2019).

Ammonium chloride is another well-known urinary acidifier, and his effectiveness as a dietary supplement has been reported in several animals species (Pollak *et al.*, 1965; Taton, Hamar and Lewis, 1984; Oetzel *et al.*, 1988; Osborne *et al.*, 1990; Kienzle and Wilms-Eilers, 1994; Funaba *et al.*, 2001; Reisinger *et al.*, 2009; Mavangira, Cornish and Angelos, 2010) and humans (Martin and Jones, 1961). Ammonium chloride challenge is also used as a diagnostic tool to distinguish proximal to distal RTA (renal tubular acidosis). Nevertheless, current diagnostic of RTA in human patients is preferably based on urinary ammonium excretion instead of urinary pH (Cook *et al.*, 2011).

In healthy dogs, oral administration of NH_4Cl results in fast and intense acidification of the urine. A dosage of 20 mg/kg PO every 8 hours (Plumb, 2005) up to 100 mg/kg BW every 12h (Mark G. Papich, 2016b) is recommended. A few studies claim that ammonium chloride effectively acidified the urine of dogs when given 200 mg/kg BW/day (Food and Authority, 2012). Groups of five dogs of multiple breeds were fed diets containing 0, 50, 100, 200, and 400 mg ammonium chloride/kg BW/day for 30 days (control group with only four dogs). Urinary pH was decreased by 200 and 400 mg ammonium chloride/kg BW/day. The group receiving 400 mg exhibited a significant decrease in blood pH and bicarbonate (Börkü *et al.*, 1996). In another study performed on eleven mature beagle dogs a single dose of 200 mg ammonium chloride/kg/BW orally, was given. Four hours following treatment, urinary pH was reduced to 5.2. (Shaw, 1989). In Senior, Merchant and Sundstrom (1984), four adult dogs (two females, two males) received orally 200 mg ammonium chloride/kg BW/day for 14 days, followed by 100 mg ammonium chloride/kg BW/day for seven days. Average daily urinary pH was kept at less than 6.0 in dogs when given 200 mg/kg BW/day and less than 6.2 when the dose was reduced to 100 mg/kg BW/day. Ammonium chloride is a systemic acidifier, the veterinary indications for his use are to acidify the urine and to help prevent and dissolve certain

types of uroliths, improve renal excretion of some toxins or drugs, and treatment of metabolic alkalosis (Plumb, 2005; Mark G. Papich, 2016a).

Ammonium chloride dissociation into chloride and ammonium ions causes the acidification properties. The liver converts the ammonium cation to urea with the release of a hydrogen ion. The hydrogen ion combines with bicarbonate to form water and carbon dioxide. In the ECF, chloride ions combine with fixed bases and decrease the alkaline reserves in the body. The net effects are reduced levels of serum bicarbonate and a decrease in blood and urinary pH (Plumb, 2005; Cook *et al.*, 2011).

In humans, the ingestion of ammonium chloride significantly increased the mean urinary output of calcium, magnesium and phosphate and has been associated with increased urine calcium content in cats and dogs (Martin and Jones, 1961; Mavangira, Cornish and Angelos, 2010). Additionally, chronic ingestion of ammonium chloride produces metabolic acidosis and alterations in calcium metabolism in humans and animals (Oetzel *et al.*, 1988; Ching *et al.*, 1990; Jones, Streeter and Goad, 2009). Nevertheless, chronic ingestion of a basal diet with 1.5% of NH_4CL for 6 months in cats did not produce osteopenia (Ching *et al.*, 1990).

Physiologic renal adaptation may develop after the supplementation of ammonium chloride, following correction of acid-base balance and decline in the urine-acidifying result exceeding 5 to 6 days (Jones, Streeter and Goad, 2009). Nevertheless, some studies have not recognized a compensation for the produced aciduria (Taton, Hamar and Lewis, 1984).

Ammonium chloride is not very palatable, and for that reason, owner compliance with daily ammonium chloride administration can be a problem. Irritation of the gastrointestinal tract, nausea, and vomiting are regularly identified following oral administration of ammonium chloride. Furthermore, if administered at high doses, it may cause hyperchloremic acidosis and hypokalemia (Plumb, 2005; Mark G. Papich, 2016a).

Ammonium chloride application is contraindicated in patients with systemic acidosis, in patients with severe hepatic disease, as ammonia may accumulate and cause toxicity (hepatic encephalopathy) and should be used carefully in patients with renal disease (Jacobs, Heimbach and Hesse, 2001; Plumb, 2005; Mavangira, Cornish and Angelos, 2010; Mark G. Papich, 2016a). For cats and dogs, 0.5 % ammonium chloride in the food can be admitted safe for an unlimited period. Higher doses of ammonium chloride in the food should be limited to veterinary treatment (Food and Authority, 2012).

b. Alkalizers

Potassium citrate is an orally administered urinary alkalizer and precursor to bicarbonate (Plumb, 2005). It has been approved in pet food in Europe since 1994 for employment as a urinary modifying substance (Stevenson *et al.*, 2000).

Results of several human medicine studies register that dietary potassium citrate supplementation given orally significantly increases urinary pH (Preminger *et al.*, 1985; Pak, Sakhaee and Fuller, 1986; Doizi *et al.*, 2018). Long-term effects of potassium citrate therapy (3240-8640 mg/day) caused a sustained increase in urinary pH in 1-5 years of treatment (Pak *et al.*, 1985; Pak, Sakhaee and Fuller, 1986; Barcelo *et al.*, 1993), although to the author's knowledge, similar studies were not performed in dogs to the date. In dogs, a dosage of 40-60 mg/kg every 8-12 hours (Stephen, Edward and Etienne, 2017) or 54 mg/kg/day (Mark G. Papich, 2016b) is recommended for an alkalinizing effect, and in one study an oral administration of up to 150 mg/kg BW/day to healthy dogs had a dose-dependent rise in urinary pH (Lulich *et al.*, 1999). Results of another study on healthy dogs indicate that administration of 150 mg potassium citrate/kg BW/day did not significantly affect mean urinary pH although did increase by 0.2 pH units with supplementation (Stevenson *et al.*, 2000).

Citrate salts are absorbed from the gastrointestinal tract and metabolized to bicarbonate. Therefore, oral intake of potassium citrate does not directly affect the amount of citrate excreted in the urine (Simpson, 1983; Stevenson *et al.*, 2000). A major determining factor for citraturia is the intracellular pH. When the intracellular pH decreases, the tubular reabsorption of citrate rises, and its excretion declines. On the contrary, when the pH increases, reabsorption of citrate drops, and its excretion rises (Meschi *et al.*, 2004). After oral administration, absorption and oxidation are promptly complete, and less than 5% is excreted unchanged. An alkaline tide is produced and a resultant increase in urinary excretion of citrate generated by an increment in citrate production inside the mitochondria of renal cells or a reduction in citrate tubular reabsorption in the proximal tubular cells (Stevenson *et al.*, 2000; Plumb, 2005).

In human medicine, it is proposed that the metabolic alkalosis results in an increase in urinary pH and an increased renal tubular reabsorption of calcium in the distal tubule, thereby decreasing its excretion in urine. However, many studies in humans indicate that citrate treatment had no effect on calcium excretion, and in dogs, a reduction in calcium excretion was not seen when dogs were given a diet supplemented with potassium citrate (Berg, Larsson and Tiselius, 1992; Stevenson *et al.*, 2000; Doizi *et al.*, 2018).

Several studies in human medicine indicate that potassium citrate supplementation significantly increases urinary pH and citrate excretion while decreasing urinary calcium oxalate supersaturation, being highly effective in preventing recurrent calcium oxalate uroliths (Berg, Larsson and Tiselius, 1992; Lulich *et al.*, 1999; Stevenson *et al.*, 2000; Meschi *et al.*, 2004).
The influence of potassium citrate supplementation on the reduction of calcium oxalate stones in veterinary patients has not been completely clarified. In dogs, oral administration of potassium citrate, although it increased urinary pH, was not associated with a consistent increase in urinary citrate excretion. Nevertheless, it is believed that dogs excrete considerably less citrate than humans (Lulich *et al.*, 1999; Stevenson *et al.*, 2000).
It is also believed that potassium citrate may be advantageous in preventing the osteopenia progression resulting from substandard acidosis (Pak, 1994; Granchi *et al.*, 2017). Potassium citrate is also used to treat hypokalemia, chronic metabolic acidosis, renal tubular acidosis, and has been listed as part of the Gonto Protocol for the treatment of Fanconi Syndrome (Gonto, 2003; Plumb, 2005; Mark G. Papich, 2016b).
The most common adverse effect is gastrointestinal disturbances still, possibly, hyperkalemia, fluid retention, and metabolic alkalosis (Plumb, 2005; Mark G. Papich, 2016b).
In humans, ingestion with a meal does not sacrifice physiological or physicochemical action (Pak *et al.*, 1991). Nonetheless, when given on an empty stomach, potassium citrate may cause minor gastrointestinal side effects (Pak *et al.*, 1991; Basiri, Taheri and Taheri, 2018).
Potassium citrate contraindications include the potential for causing hyperkalemia, which can lead to cardiovascular toxicity and muscular weakness, aluminum toxicity, and acute kidney injury (Plumb, 2005; Mark G. Papich, 2016b). In humans, it has been observed that through long-term citrate therapy in patients with functioning kidneys, abnormal total body retention of aluminum does not occur (Pak, 1994; Sakhaee *et al.*, 1996).
In patients with delayed gastric emptying conditions, esophageal compression, intestinal obstruction or constriction, potassium citrate tablets are contraindicated (Plumb, 2005; Mark G. Papich, 2016b). Nonetheless, humans prescribed potassium citrate had a higher health-related quality of life and were less probable to report nausea, stomach upset, or cramps opposed to those not prescribed (Raffin *et al.*, 2018).

Calcium carbonate is a calcium supplement used for oral administration to treat hyperphosphatemia in patients with chronic kidney disease and as a calcium supplement in animals with chronic hypocalcemia, occasionally used with vitamin D supplements (Biasibetti

et al., 2018). It could also be used as an antacid to treat gastric hyperacidity and GI ulcers but is not usually prescribed for this purpose in small animals (Plumb, 2005; Mark G Papich, 2016). A dosage of 70-185 mg/kg BW/day (Mark G Papich, 2016) and 90-150 mg/kg BW/day (Plumb, 2005) given with food PO is recomended.

Calcium carbonate is known to be poorly absorbed, when given with meals less than 50% is absorbed, it binds to dietary phosphorus and forms an insoluble compound (calcium phosphate) that is eliminated in the feces (Stevenson, Hynds and Markwell, 2003; Plumb, 2005; Mark G Papich, 2016).

Calcium carbonate does not have many side effects. Hypercalcemia and extra-osseous (soft tissue) calcification are the primary concerns associated with calcium carbonate, mainly when using high dosages long-term (Plumb, 2005; Mark G Papich, 2016).

Calcium salts should not be administered when hypercalcemia is present and in animals predisposed to forming calcium-containing uroliths. When used to prevent hyperphosphatemia, caution is recommended to avoid hypercalcemia in patients with renal failure. Like any calcium supplements, constipation and intestinal bloating can occur (Plumb, 2005; Mark G Papich, 2016).

Chapter III

Practical section

1. *Internship Report*

The final practical internship of my Veterinary Medicine Master included three different periods:

The first, from September the 2nd until October the 29th, was an internship at the Hospital Veterinário do Baixo Vouga. The internship included the following functions: consultation assistance; surgery assistance; anesthesiology assistance; assistance in taking x-rays; ultrasound examination assistance; serum biochemistry and blood sample measuring equipment's; IV catheter placement; assembling fluid therapy systems; SC, IV, IM, PO drug administration.

The second, from November the 4th until December the 20th of 2019, was a 7-week clinical rotation program at Utrecht University, in the Netherlands. This rotational program included specialties such as: Internal Medicine, Cardiology, Haematology, Endocrinology, Reproduction, Dermatology, Intensive Care, Imagiology, Anesthesiology and Surgery.

The third, with a duration of 31 days, was spend with the practical work of one study of a major clinical research project, entitled "Effects of diets and supplements on the urinary pH in dogs". The study was also performed at Utrecht University, in the Netherlands.

Effects of diets and supplements on the urinary pH in dogs

2. Introduction

Urinary pH is a rough but helpful estimate of acid-base balance (Barsanti, 2012). Urinary pH is not constant and may fluctuate over the 24 hours. There is an indication of a physiologic acid-base rhythm with a curve of diurnal fluctuation which is characteristic for a given individual, and it is a result of several factors such as diet, emotional status, exercise, personality and pulmonary ventilation (Elliot, Sharp and Lewis, 1959). The physiological process regarding the alkalization of the blood and urine, *i.e.* rise in the pH, following a meal is designated the alkaline tide effect (Niv and Fraser, 2002).

Food and endogenous metabolic processes are the sources of acid or base intake or production, and so it is possible to efficiently alter or adjust the urinary pH by solely dietetic means (Dwyer *et al.*, 1985; Remer and Manz, 1994). Urinary pH manipulation is beneficial in multiple

conditions but is particularly important in some types of urolith formation, considering urine acidity or alkalinity influences what type of mineral precipitates (Gleaton, Bartges and Laflamme, 2001). Numerous therapeutic diets are formulated to prevent recurrence of uroliths in dogs. The potential of a diet to acidify/alkalize the urine depends on its ingredients and the equilibrium between acidifiers, such as methionine, calcium sulfate and ammonium chloride, or alkalizers, such as calcium carbonate and potassium citrate (Queau, 2019).

3. Research question

Can dogs urinary pH be manipulated by the oral supplements: potassium citrate and a ammonium chloride containing solution (Urical) or by the therapeutic dry foods: Hill's® Prescription Diet® u/d® Canine (u/d) and Royal Canin Urinary SO dog (Urinary SO)? Does food intake cause a transitory increase in pH (alkaline tide)?

4. Hypothesis

The u/d diet (MER, kcal/day = 1.6 x 70 $BW^{0.75}$), fed over two meals per day, or potassium citrate (130-211 mg/kg BW/day), given two times daily with food, will cause a significant increase ($P<0.05$) in the dog's urinary pH when compared with baseline.

The Urinary SO diet (MER, kcal/day = 1.6 x 70 $BW^{0.75}$), fed over two meals per day, or Urical (5 ml/10kg BW/day), given 2 times daily with food, will cause a significant decrease ($P<0.05$) in the dog's urinary pH when compared with baseline.
Food intake causes a transitory increase in pH (alkaline tide).

5. Objetives

- To assess the effect of the oral supplementation of potassium citrate and an ammonium chloride solution on dog's urinary pH;
- To access the effect of Urinary SO and u/d diets on dog's urinary pH;
- To evaluate the postprandial alkaline tide effect in dogs.

6. Materials and Methods

Animals

Seven Beagle research dogs from Utrecht University were randomly selected to be a part of the study. All dogs were male and intact with ages between 1 and 5 years old and weighing 11.3 ± 2.15 kg (±SD), individual dog charateristics can be seen in **Table 1**. Based on physical examination, complete blood count and serum biochemistry, the dogs were determined to be healthy.

Table 1: Dog characteristics.

Dog Name (#)	Age, years	Weight, kg
Antonio (1)	5	15.4
Austin (2)	4	9.7
Denver (3)	4	9.5
Houston (4)	4	10.4
Lyon (5)	1	10.1
Stitch (6)	2	13.9
Texas (7)	4	10.4

Study design

The study was conducted over a period of 31 days. During this period all seven dogs participated in four different trials. Each trial had a length of 2-5 days and held parted by a 3-4-day washout interval.

During the washout interval, the dogs received solely a dry commercial diet, Hill's Science Plan Adult Medium Breed Advanced Fitness Lamb and Rice[(a)], once a day at 07h00. Food allowance was calculated according to the adult maintenance energy requirements MER = 1.6 x 70 $BW^{0.75}$ (Hand *et al.*, 2010), and water was provided *ad libitum*.

In the first week, urine samples were collected from dogs 1 and 6, every two hours between 07h00 and 15h00 for five consecutive days, with an additional measurement at 17h00 on the first day and at 10h00 on the last four days. This results aid to decide the best time points for urine samples collection, while already collecting the urinary pH baseline data for these two dogs. The samples for the urinary pH baseline of the remaining dogs were collected before Trial 2, during only one day and using less time points (see below).

Trial 1 had a total duration of five days. During the first three days of trial 1, all dogs were fed a dry standard commercial diet, Hill's Science Plan Adult Medium Breed Advanced Fitness Lamb and Rice[(a)], supplemented with potassium citrate twice daily, at 07h00 and 15h00 (**Figure 3**). Food allowance was calculated according to the adult maintenance energy MER = 1.6 x 70 $BW^{0.75}$ (Hand *et al*., 2010), and water was provided *ad libitum*. During the last two days of trial 1, the dogs were fed at the same time point, but the morning supplement instead of being given with the food was given at 10h30 (**Figure 4**). Dogs 1, 2, 3, 4, 6 and 7 were supplemented with four capsules/day, and dog 5 received two capsules/day, 500 mg of potassium citrate was supplied per capsule (**Figure 5**).

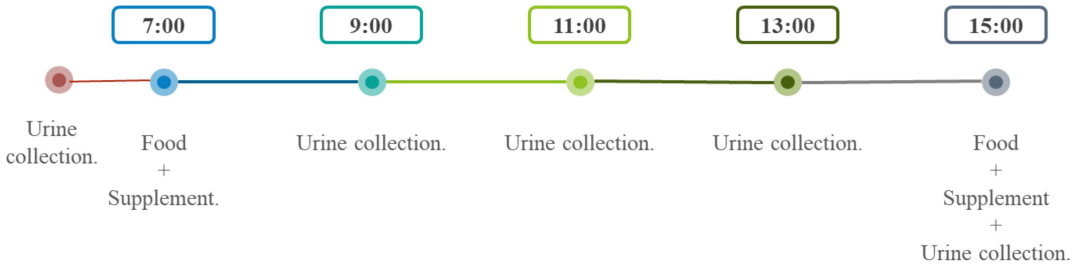


Figure 3: Timeline for trial 1 - part 1 and trial 2

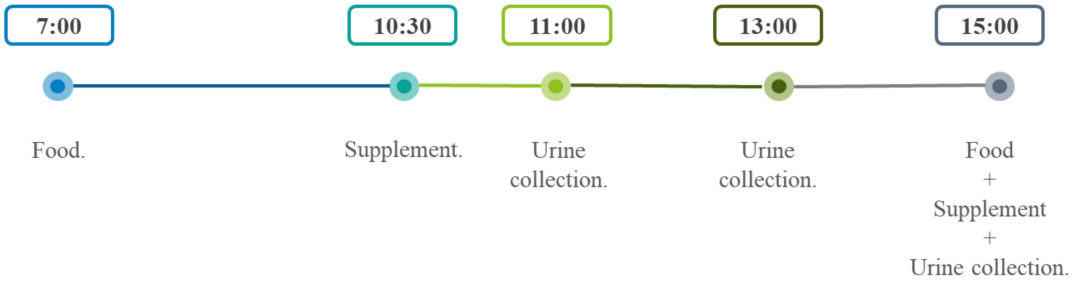


Figure 4: Timeline for trial 1 - part 2

A 3-day washout period was performed. The day before the start of the second trial, a one-day baseline measurement of urinary pH was collected every two hours between 07h00 and 15h00, for the remaining five dogs (2, 3, 4, 5 and 7).

Trial 2 had a total duration of two days. The dogs were fed two times daily a dry standard commercial diet, Hill's Science Plan Adult Medium Breed Advanced Fitness Lamb and Rice[(a)], with Urical (5ml/10kg BW day) mixed with the food (**Figure 5**). Food allowance was calculated according to the adult maintenance energy requirements MER = 1.6 x 70 $BW^{0.75}$ (Hand *et al.*, 2010), and water was provided *ad libitum* (**Figure 3**).
A 3-day washout was performed between the second and the third trial.

Figure 5: Supplements used in the study. On the left, the Urical solution and on the right, the potassium citrate supplement.

Trial 3 had a total duration of three days. The dogs were fed two times daily a therapeutic diet, Hill's™ Prescription Diet™ u/d™ Canine Hill's Pet Nutrition[(b)] that is, according to the supplier specifications, formulated to induce an alkaline urine. Food allowance was calculated according to the adult maintenance energy requirements MER = 1.6 x 70 $BW^{0.75}$ (Hand *et al.*, 2010), and water was provided *ad libitum*. Urine was collected during the last two days, every two hours between 07h00 and 15h00 (**Figure 6**).

Figure 6: Timeline for trial 3 and 4

A 4-days washout was made between the third and the fourth trial.

Trial 4 had a total duration of three days. The dogs were fed two times daily a therapeutic diet, Royal Canin Canine Urinary SO, Royal Canin[(c)], formulated to induce an acidic urine. Food allowance was calculated according to the adult maintenance energy requirements MER = 1.6 x 70 $BW^{0.75}$ (Hand *et al.*, 2010), and water was provided *ad libitum*. Urine was collected during the last two days every two hours between 07h00 and 15h00 (**Figure 6**).

Urinalysis

All urinalysis results were obtained from urine samples collected spontaneously during a walk (**Figure 7**). The urine samples were stored in an eppendorf tube (**Figure 8**), briefly, at room temperature (approx. 18°C) until analysis, which was performed within 3 hours after sample collection.

The urine samples were first centrifugated and then analyzed at the Utrecht University laboratory for: pH, specific gravity, leucocytes, nitrite, urobilinogen, protein, blood, ketone, bilirubin, and glucose.

PH estimation

Urinary pH was estimated using the following equations (Yamka and Mickelsen, 2006):

Urinary pH (wet and dry foods) = 7.30 + (0.54 × sodium) + (0.63 × potassium) - (0.53 × chloride) - (1.67 × sulfur) - (0.61 × phosphorus) + (2.07 × cystine)

Urinary pH (dry foods) = 8.09 - (1.15 × sulfur) - (0.50 × phosphorus) - (0.16 × methionine)

The results were calculated based on the information available on the diet package; therefore, specific parameters which were not present were considered zero.

Figure 7: Free walk urine collection

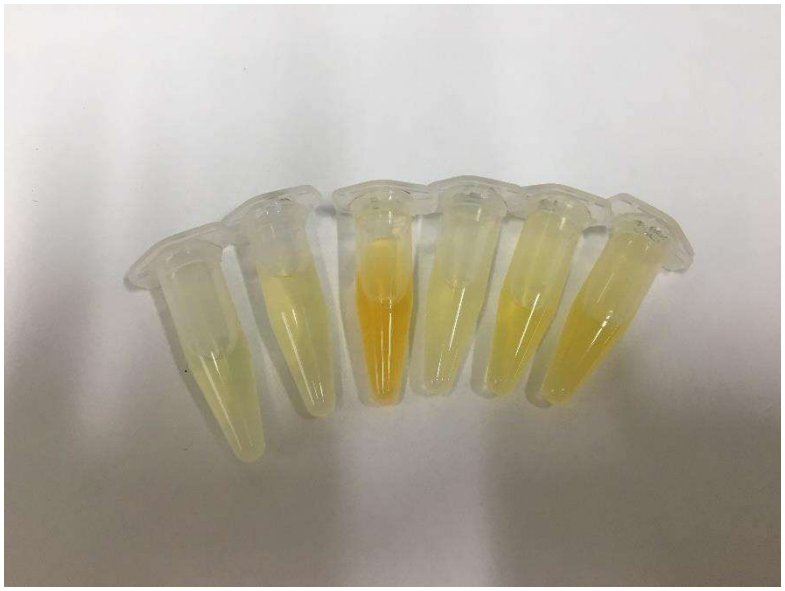


Figure 8: Urine samples in eppendorf tubes

The pH of the urine samples was determined using a pH meter (HI 2209 pH meter- Hanna instruments), a pH paper (Machery-Nagel, duotest 5.0-8.0; **Figure 9**) and a urine strip (Arkray manufacturer A.Menarini diagnostics Aution sticks 10EA; **Figure 10**) along with an automatic measurement device (Aution micro PU-4010 from Arkray manufacturer A.Menarini diagnostics). Refractometry was used to determine the USG. The urine strips (Arkray manufacturer A.Menarini diagnostics Aution sticks 10EA) were also used to determine leucocytes, nitrite, urobilinogen, protein, blood, ketone, bilirubin, and glucose.

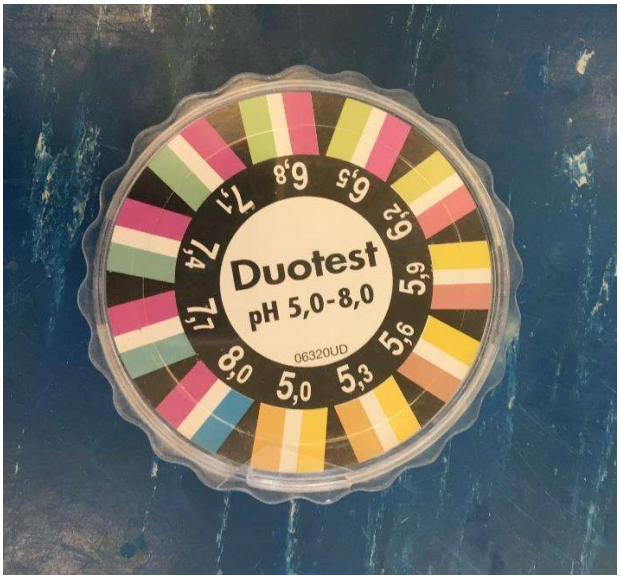


Figure 9: Urine pH paper (Machery-Nagel, duoteste 5.0-8.0)

Figure 10: Urine pH strip (Arkray manufacturer A.Menarini diagnostics Aution sticks 10EA)

Statistics

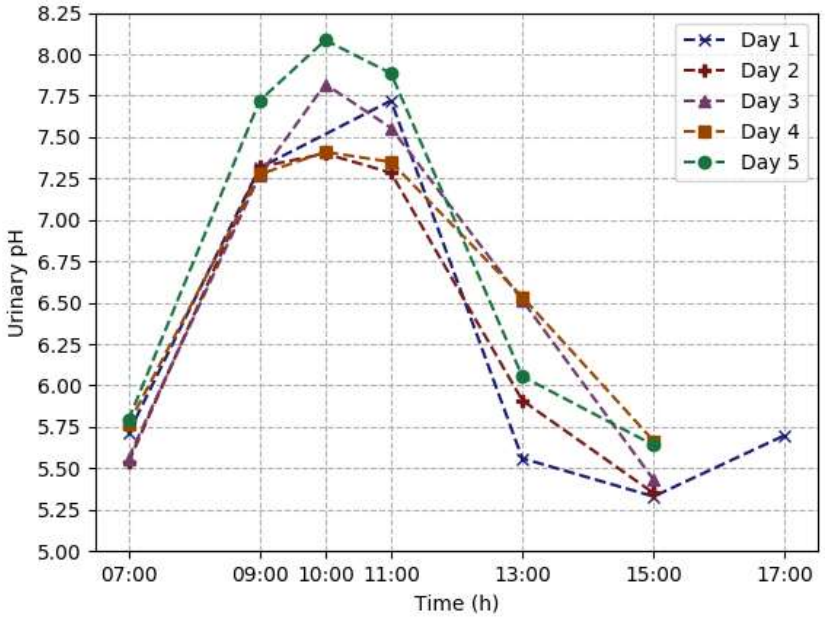
The pH results were expressed as mean ± standard deviation (SD). The data were analyzed using the One-Way Analysis of Variance F Test method. The post-hoc Tukey test was performed to identify significant differences between treatment means at a significance level of 0.05. The Pearson's coefficient of correlation was used to evaluate the correlation between the different urinary pH measurement methods. A correlation >0.75 was considered a strong correlation. A difference of at least 0.5 on urinary pH was considered a clinically relevant difference. All data were analyzed in JMP 7 ® (SAS institute) software. Graphic representations were created using a plotting library called Matplotlib for the Python 3.6 programming language and its numerical mathematics extension NumPy.

7. Results

All urinary parameters (specific gravity, leucocytes, nitrite, urobilinogen, protein, blood, ketone, bilirubin, and glucose) besides pH remained within the reference ranges for clinically healthy dogs.

Urinary pH

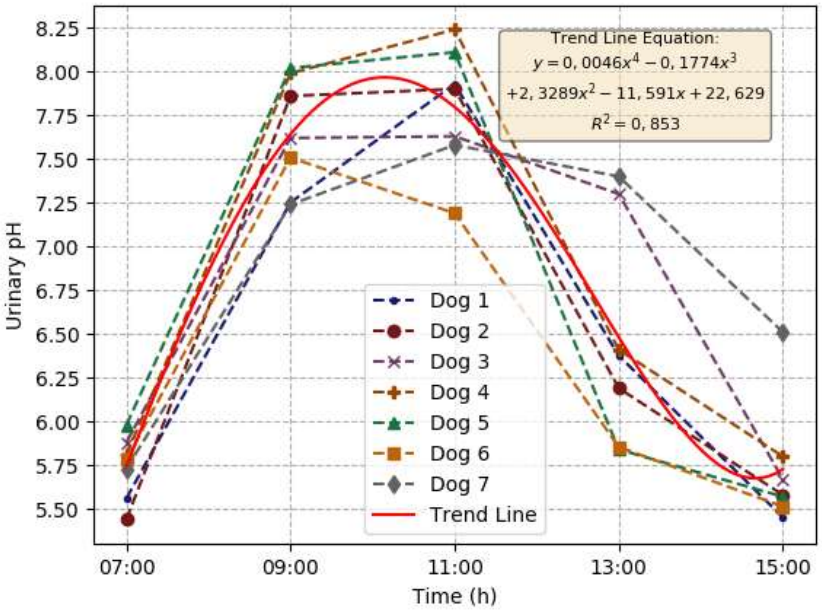
The baseline urinary pH mean values per day and per time point obtained with the dogs 1 and 6 are represented in **Graph 1.**



Graph 1: Baseline urinary pH values for dogs 1 and 6, when fed the control diet divided over 2 meals per day. Time effect: P=0.019; Day effect: P=0.571.

There was no significant difference in urinary pH between days (P=0.571). It was observed a significant difference in urinary pH between: 07h00 and 09h00 (P<0.001), 11h00 and 13h00 (P<0.001) and 13h00 and 15h00 (P=0.011). There was no significant difference in urinary pH between: 09h00, 10h00 and 11h00 (P=0.424), or 15h00 and 17h00 (P=0.185).

The mean diurnal variation in urinary pH for all dogs and the mean urinary pH for baseline is represented in **Graph 2**.



Graph 2: Mean diurnal urinary pH variation for all dogs between 07h00 and 15h00.

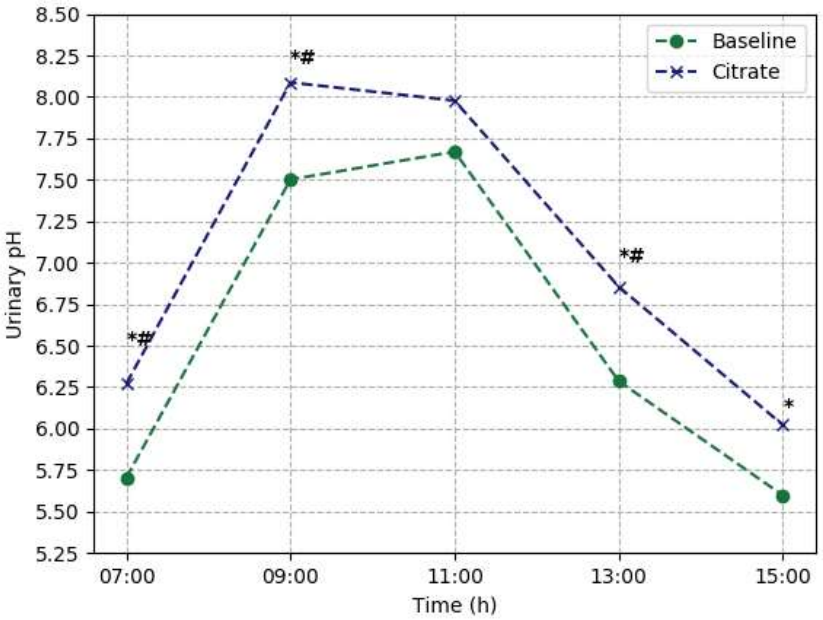
The mean urinary pH ± standard deviation (SD) between 7h00 and 15h00 for the baseline and the two alkalize treatments, potassium citrate supplementation and u/d diet, are presented in **Table 2**.

Table 2: Mean urinary pH (± SD) between 7h00 and 15h00 for baseline, potassium citrate supplement (*trial 1- part 1*) and u/d diet (*trial 3*).

Time (h)	Baseline	Potassium citrate	u/d	P-value
7	$5.70 \pm 0.213^{a, x}$	$6.27 \pm 0.555^{b, x}$	$5.93 \pm 0.407^{a, b, x}$	**P=0.001**
9	$7.50 \pm 0.354^{a, y}$	$8.09 \pm 0.263^{b, y}$	$8.16 \pm 0.631^{b, y}$	**P<0.001**
11	$7.67 \pm 0.507^{a, y}$	$7.98 \pm 0.318^{a, b, y}$	$8.15 \pm 0.442^{b, y}$	**P=0.011**
13	$6.29 \pm 0.705^{a, z}$	$6.86 \pm 0.561^{b, z}$	$7.45 \pm 0.476^{c, z}$	**P<0.001**
15	$5.60 \pm 0.307^{a, x}$	$6.02 \pm 0.453^{b, x}$	$6.14 \pm 0.702^{b, x}$	**P=0.012**
P-value	**P<0.001**	**P<0.001**	**P<0.001**	

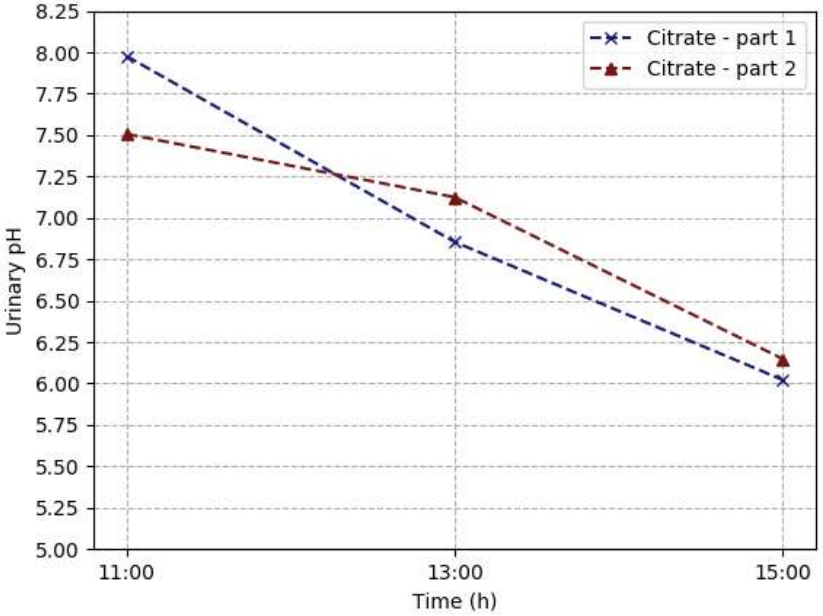
SD, standard deviation.
a, b and c, different letters in the same line indicate statistical significance between trials, P<0.05.
x, y and z, different letters in the same column indicate statistical significance between time points for the designated trial, P<0.05.

Compared with baseline, the potassium citrate supplement increased significantly the mean urinary pH (P<0.001, *Trial - part 1*). Significant differences were observed at 7h00 (P<0.001), 9h00 (P<0.001), 13h00 (P=0.012) and 15h00 (P=0.004). A clinically relevant difference of at least 0.5 pH units was observed at 7h00, 9h00 and 13h00 (**Graph 3**).



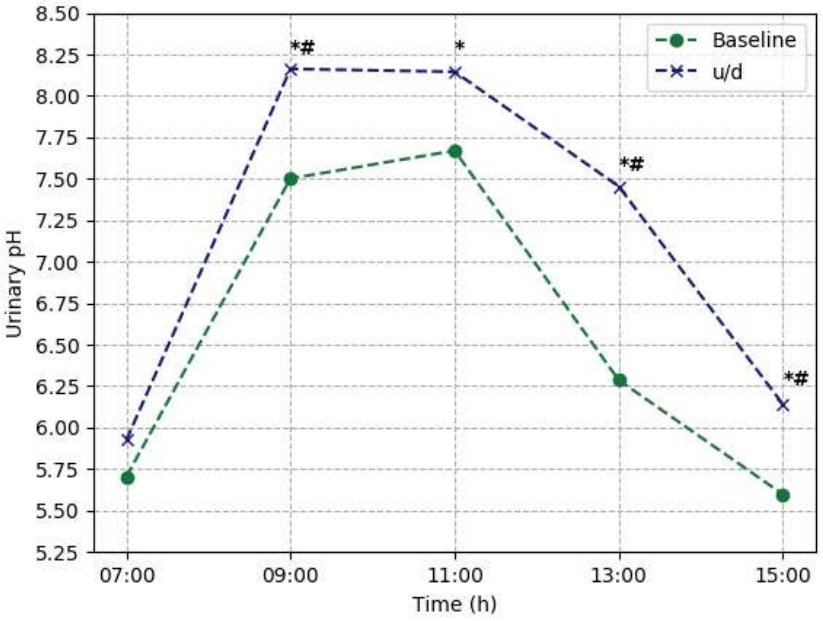
Graph 3: Mean diurnal urinary pH of baseline and *trial 1 - part 1.* P<0.001
* means per time point are significantly different (P<0.05)
# means per time point differ from the baseline at least 0.5 pH units'

The morning supplement being given with the food at 07h00 (*trial 1 - part 1*) or at 10h30 (*trial 1 - part 2*) did not have a significant effect on urinary pH (P=0.819), which can be observed in **Graph 4**.



Graph 4: Mean diurnal urinary pH of potassium citrate supplement (*trial 1*) part 1 and part 2. P=0.819

In **Graph 5** it can be observed that in Trial 3, the intake of u/d diet resulted in significantly higher urinary pH when in comparison to the baseline (P<0.001). The difference was statistically detected at 9h00 (P=0.002), 11h00 (P=0.012), 13h00 (P<0.001) and 15h00 (P=0.011) and was clinically relevant (≥ 0.5 pH units) at 9h00, 13h00 and 15h00.



Graph 5: Mean diurnal urinary pH of baseline and u/d diet (*trial 3*). P<0.001
* means per time point are significantly different (P<0.05)
# means per time point differ from the baseline at least 0.5 pH units'

The mean urinary pH ± standard deviation (SD) between 7h00 and 15h00 for baseline and the two acidifying treatments is represented in

Table *3*.

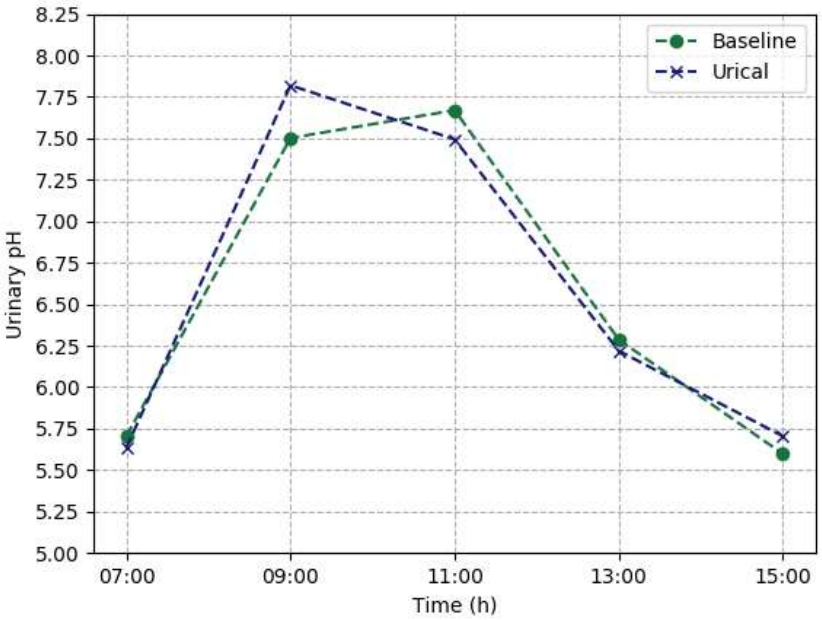
Table 3: Mean urinary pH (± SD) between 7h00 and 15h00 of baseline, Urical solution (*trial 2*) and Urinary SO diet (*trial 4*).

Time (h)	Baseline	Urical	Urinary SO	P-value
7	$5.70 \pm 0.213^{a, x}$	$5.63 \pm 0.267^{a, x}$	$5.31 \pm 0.310^{b, x}$	**P<0.001**
9	$7.50 \pm 0.354^{a, y}$	$7.82 \pm 0.639^{a, y}$	$6.16 \pm 0.897^{b, y}$	**P<0.001**
11	$7.67 \pm 0.507^{a, y}$	$7.49 \pm 0.342^{a, y}$	$6.05 \pm 0.260^{b, y}$	**P<0.001**
13	6.29 ± 0.705^{z}	6.22 ± 0.405^{z}	$5.89 \pm 0.312^{y, z}$	**P=0.094**
15	5.60 ± 0.307^{x}	5.70 ± 0.348^{x}	$5.46 \pm 0.295^{x, z}$	**P=0.144**
P-value	**P<0.001**	**P<0.001**	**P<0.001**	

SD, standard deviation.
a, b and c, different letters in the same line indicate statistical significance between trials, P<0.05.

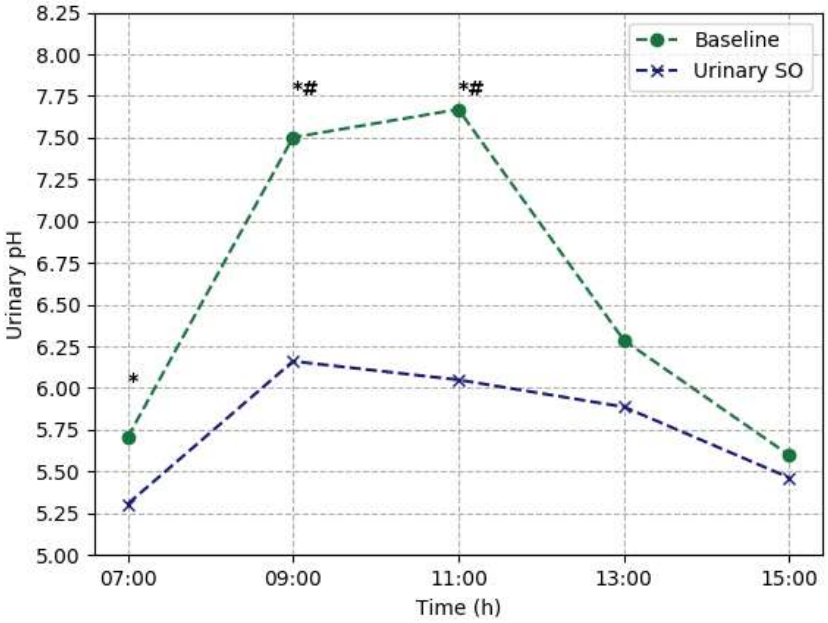
x, y and z, different letters in the same column indicate statistical significance between time points for the designated trial, P<0.05.

Regarding the acidifying treatments, the treatment group receiving the Urical solution (*trial 2)* did not have a significant effect on mean urinary pH (P=0.828) when compared to the baseline (**Graph 6**).



Graph 6: Mean diurnal urinary pH of baseline and Urical solution (*trial 2*). P=0.828

The treatment group receiving the Urinary SO diet (*Trial 4)* had a significantly lower urinary pH when in comparison to the baseline (P<0.001). The difference was statistically detected at 7h00 (P<0.001), 9h00 (P<0.001) and 11h00 (P<0.001), with a relevant difference of at least 0.5 pH units at 09h00 and 11h00 (**Graph 7**).



Graph 7: Mean diurnal urinary pH of baseline and Urinary SO diet (*trial 4*). P<0.001
* means per time point are significantly different (P<0.05)
# means per time point differ from the baseline at least 0.5 pH units'

In Graphs 1, 2, 3, 5, 6 and 7 an overall increase in urinary pH between 9h00 and 11h00 (approximately 2-4 hours after meal) is evident in all four treatments and baseline measurements.

PH meter *vs.* pH strip

There was close agreement between the reference method (pH meter) and the other two measurement methods: urine pH paper (Machery-Nagel, duoteste 5.0-8.0) and the urine pH strip (Arkray manufacturer A.Menarini diagnostics Aution sticks 10EA). The Pearson's coefficient of correlation was 0.92 for the urine pH paper and 0.97 for the urine strip.

8. Discussion

The purpose of the study was to assess the effect of the oral supplementation of potassium citrate and an ammonium chloride solution, along with two therapeutic diets, Urinary SO and u/d, on dog's urinary pH, and to evaluate the postprandial alkaline tide effect in dogs. Even though the solution containing ammonium chloride (Urical) did not produce a significant urinary pH difference, results indicated that the oral supplement potassium citrate and the therapeutic dry foods, Hill's ® Prescription Diet® u/d® Canine (u/d), and Royal Canin Urinary S/O dog (Urinary S/O), can manipulate urinary pH.

Study design

The urinary pH is a parameter subject to daytime fluctuation and variation between days. To access the significance of these variations and to decide the most adequate measurement points for the trial, preliminary results were collected in only two dogs for five consecutive days. The consecutive baseline measurements - every two hours between 7h00 and 15h00, with an additional measurement at 17h00 on the first day and at 10h00 on the last four days – were made. Since no significant differences ($P<0.05$) in mean urinary pH were observed between days, only a one-day baseline measurement was taken for the other five dogs and the urine samples were collected every 2 hours between 07h00 and 15h00. The 10h00 measurement was not performed since the logistics required for this measurement were not justified by the scarcity of significant differences in urinary pH between 09h00, 10h00 and 11h00. The 17h00 measurement was likewise not performed since there was no significant difference ($P<0.05$) between the 15h00 measurement.

Postprandial alkaline tide

Factors including diet, emotional status, exercise, and pulmonary ventilation are recognized to affect urinary pH in humans (Murayama and Taguchi, 1993), and the likewise is thought to occur in dogs. There is no reason to assume a significant difference in emotional status, exercise, and pulmonary ventilation among the dogs in the present study. The diet effect was one of the aspects under direct evaluation in our study.

Our results show that at baseline and in all treatments, it was observed an increase in urinary pH between 9h00 and 11h00 (approximately 2-4 hours after food intake). This is no surprise, as similar pattern has been observed in other studies performed in dogs (Stevenson *et al.*, 2000; Stevenson and Markwell, 2001). The postprandial alkaline tide results from the secretion of gastric acid in response to the food ingestion (Brooks, 1985). As a result of the acid "loss" the kidneys compensate by conserving acid, which consequently produces an alkaline urine (Finke and Litzenberger, 1992).

Alkalizing treatments

Potassium citrate is used for urinary alkalization and treatment of chronic metabolic acidosis and has a quick and temporary effect on systemic acid-base status (Papich, 2016b), therefore a two/three-day trial was enough to evaluate the urinary pH effect.

Results of several human medicine studies show that dietary potassium citrate supplementation given orally significantly increases urinary pH (Preminger *et al.*, 1985; Pak, Sakhaee and Fuller, 1986; Doizi *et al.*, 2018). In dogs, a dosage of 40-60 mg/kg every 8-12 hours is recommended for an alkalinizing effect (Stephen, Edward and Etienne, 2017) and an oral administration of up to 150 mg/kg BW/day to healthy dogs resulted in a dose-dependent rise in urine pH (Lulich *et al.*, 1999). Results of another study on healthy dogs indicate that administration of 150 mg potassium citrate/kg BW/day increased mean urinary pH by 0.2 pH units. Nonetheless, this increase was not statistically significant (Stevenson *et al.*, 2000). In the present study, the average dosages of 130-211 mg/kg BW/day (2-4 capsules per day to dogs with different BW) increased effectively ($P<0.001$) the mean urinary pH.

In human literature, the effects of long-term potassium citrate therapy (3240-8640 mg/day) caused a sustained increase in urinary pH during 1-5 years of treatment (Pak *et al.*, 1985; Pak, Sakhaee and Fuller, 1986; Barcelo *et al.*, 1993). To the author's best knowledge, similar studies were not performed in dogs to date.

To further study the potassium citrate supplementation effect, an additional trial was performed (*trial 1 - part 2*). In the first part of the trial the potassium citrate was given twice daily, at 07h00 and at 15h00 with the meal, while in the second part, instead of being given with the morning

meal it was given at 10h30. This second part of the trial aimed to access the effect of the potassium citrate supplementation independently from the food intake and to evaluate its capacity to prolong the alkaline-tide effect by administrating it at the peak of the tide.

It is suggested that following the administration of potassium citrate, an alkaline tide is produced and a resultant increase in urinary excretion of citrate generated by an increment in citrate production inside the mitochondria of renal cells or a reduction in citrate tubular reabsorption in the proximal tubular cells (Stevenson *et al.*, 2000; Plumb, 2005). It was then expected that after the administration of potassium citrate at 10h30 the urinary pH would continue high since the kidney counteracts the resulting metabolic alkalosis with increased excretion of alkaline ions (HCO_3^-) through the urine (Rune, 1965, 1966; Brooks, 1985; Niv and Fraser, 2002). However, this was not observed since there was not a significant difference in urinary pH compared to when given at 07h00, as illustrated in Graph 4. It can be speculated that food intake influences the potassium citrate supplementation. Nevertheless, the effect of meals on the physiological and physicochemical actions of potassium citrate was investigated in humans with nephrolithiasis and a constant dietary regimen supplemented with potassium citrate (2160 mg, three times per day), whether given with food or on an empty stomach, and the result was a significant increased ($P<0.05$) in the urinary pH (Pak *et al.*, 1991).

In the present study, measurements of mean urinary pH after 17h00 were not performed, but it can be expected that a second smaller peak will follow the second meal considering a small rise in mean urinary pH is evident at 17h00 after the 15h00 evening meal (Graph 1). Stevenson *et al.* (2000) observed a smaller alkaline tide effect following the 15h00 meal, and that diets containing potassium citrate kept a higher urinary pH than the control diet, from 15h00 to 21h00. Stevenson *et al.* (2000) did not explain the mechanisms behind this smaller alkaline tide. It can be speculated that the second alkaline tide is smaller due to daytime variations, urinary pH is regularly higher in the daytime and lower in the early morning and at night (Murayama and Taguchi, 1993). It is also possible that the production of bicarbonate, subsequent to the production of HCl is smaller on the second meal of the day and, consequently, so will be effect on urinary pH. Urinary pH being regularly higher in the daytime and lower in the early morning and at night, a single evening dose of potassium citrate could be helpful to balance the acidity during the 24h day period. To evaluate this effect, and to assess more acidity times of the day,

it would be necessary to monitor the urinary pH over the 24 hours. Unfortunately, the logistics necessary for this amount of measurements were impossible to apply in practice.

The u/d diet was formulated to offset metabolic acidosis, produce alkaluria due to its composition including potassium citrate and calcium carbonate and it is supposed to reach a target urinary pH between 7.1 and 7.7 when fed to dogs. In the present study, it was observed a mean urine pH of 7.2. Applying Yamka, Friesen and Schakenraad (2006) equations for the estimation of urinary pH, a result of 7.2 was obtained with the wet and dry food equation and 7.8 with the dry food equation. Thus, the results obtained from the equations were in agreement with the diet's target urinary pH. Nevertheless, there was some potential sources of inaccuracies. In the present study, the formula was calculated based on the information available on the diet package, and so, specific parameters which were not present, were considered zero. Nutrient analysis of the treatment diets would have been preferred for accuracy. Notwithstanding the fact that the study design based on which the equation was formulated accounts for some controversy regarding the accuracy and validity of these estimations (see for detailed discussion Chapter I - 4. Urinary pH estimation).

In a case report, a dog with calcium oxalate uroliths received as part of the treatment a combination of a canned and dry formulation of u/d (Lulich *et al.*, 1999). In this study, before the institution of the diet the mean urinary pH was 6.5, and after 35 days of treatment, the mean urinary pH was 8.5. At the end of day 531, mean urinary pH was still 8.5 (Lulich *et al.*, 1999). In our study, only a three-day trial was performed with a significantly increased urinary pH observed already in day one. Thus, the diet effect on urinary pH can be evaluated in short term studies.

In the present study, the group receiving the u/d diet had a higher mean urinary pH for a longer period compared to the trial group receiving the potassium citrate supplement (**Table 2**). They each contain potassium citrate, but the u/d diet contains an extra alkalize agent, the calcium carbonate. Kienzle *et al.* (1994) observed that with the calcium carbonate diet (with a base excess of +305), the postprandial alkaline tide persisted longer when compared to the other diets, and hypothesized it was due to the lower solubility of the calcium carbonate, thus being absorbed rather slowly (mainly in the large bowel). This effect could also be responsible for our observations, although the present study was not designed to determine the impact of any

specific dietary element on urinary pH but the diet impact as a whole. Consequently, the results were influenced by potential interactions between several nutritional components and internal metabolic factors.

Acidifying treatments

A few studies claim that ammonium chloride effectively acidified the urine of dogs when given 200 mg/kg BW/day (Food and Authority, 2012). Groups of five dogs of multiple breeds were fed diets containing 0, 50, 100, 200, and 400 mg ammonium chloride/kg BW/day for 30 days (control group with only four dogs). Urinary pH was decreased by 200 and 400 mg ammonium chloride/kg BW/day. The group receiving 400 mg exhibited a significant decrease in blood pH and bicarbonate (Börkü *et al.*, 1996).

In another study performed on eleven mature beagle dogs, a single dose of 200 mg ammonium chloride/kg/BW was given orally. Four hours following treatment, urinary pH was reduced to 5.2 (Shaw, 1989). In Senior, Merchant and Sundstrom (1984) four adult dogs received orally 200 mg ammonium chloride/kg BW/day for 14 days, followed by 100 mg ammonium chloride/kg BW/day for seven days. Average daily urinary pH was kept lower than 6.0 in dogs given 200 mg/kg BW/day and lower than 6.2 when the dose was reduced to 100 mg/kg BW/day.

The small number of studies available, together with controversial results, judges for an important level of uncertainty (Food and Authority, 2012). Nevertheless, it is accepted that the oral administration of ammonium chloride induces acidic urine and a dosage of 100 mg/kg BW every 12h is recommended (Mark G. Papich, 2016b).

In the present study, an ammonium chloride containing solution – Urical – was chosen. Urical is a liquid food supplement for dogs and cats that offers support for bladder stones or urinary crystals in the urinary tract, with a target urinary pH <6.5. A dosage of 5 mL/10kg BW/day of Urical (amount indicated by the manufacturer) was used. This dosage was not effective in lowering the urinary pH. It is possible that the amount (mg/kg BW/day) of ammonium chloride present in the solution was not enough to produce a significant urinary pH variation. Unfortunately, it was impossible to know the exact amount of ammonium chloride present in

the solution since the manufacturer did not provide that information. A clinical analysis of the solution could have overcome this issue.

Ammonium chloride has a bitter taste when added to food (Mark G. Papich, 2016a). Six healthy adult cats receiving ammonium chloride with food, reported a mild apathy before the food was finally refused. One animal showed intense vomiting (Kienzle and Wilms-Eilers, 1994). In the present study, there was no witnessed vomits, but the administration of the supplement was not easy as the dogs tried to spit the supplement and drooled a lot, being noticeable the unpalatability of this treatment.

The Urinary SO diet is marketed to aid prevention of struvite urolith formation in dogs and to reach a target urinary pH between 6 and 6.5. This diet contains two urinary acidifying substances, calcium sulfate and DL-methionine. In dogs, the addition of calcium sulfate followed a small, but not significant, urinary acidification (Janczikowski, Wolf and Kamphues, 2008; Halfen *et al.*, 2018). However, multiple studies verify the effectiveness of L-methionine/DL-methionine as a urinary acidifier in both humans, cats, and dogs (Funaba *et al.*, 2001; Jacobs, Heimbach and Hesse, 2001; Hickey, Son and Wismer, 2015; Siener, Struwe and Hesse, 2016; Halfen *et al.*, 2018). The present study was not designed to determine the impact of any nutrient or dietary element on urinary pH but the diet impact as a whole. Consequently, the results were influenced by potential interactions between several nutritional components and internal metabolic factors.

In the present study, the mean urinary pH observed when the dogs were fed the SO diet was 5.77. Applying Yamka, Friesen and Schakenraad (2006) equations for the estimation of urinary pH, a result of 5.9 and 7 to the wet and dry food equation and to the dry food equation, respectively, were obtained. The results from the equations and from the present study were not in agreement with the diet's target urinary pH. As mentioned earlier, it has to be kept in mind that there are several sources of inaccuracies and variations involved in the estimation of urinary pH from the dietary analysis. In the present study, the formula was calculated based on the information available on the diet package, and so, specific parameters which were not present, were considered zero. That could explain the differences obtained between the results from the equations and the diet's target urine pH. Nutrient analysis of the treatment diets would have been more accurate. Notwithstanding the fact that the study design based on which the equation

was formulated accounts for some controversy regarding the accuracy and validity of these estimations (see for detailed discussion Chapter I – 4. Urinary pH estimation). As stated before, factors including diet, emotional status, exercise, and pulmonary ventilation are thought to affect urinary pH in dogs (Murayama and Taguchi, 1993). This factors, excluding the diet, could be behind the reason for the discrepancy between the results observed in our study and the diets target urinary pH, since the diet effect is one of the aspects under direct evaluation in our study, and in this case was the same.

pH meter *vs*. pH strips

Considering clinically relevant disparities occur with an unacceptable frequency, it is suggested that the reagent strips may only be applied when obtaining pH approximations for routine urinalysis but are not recommended when regular and accurate pH measurements are critical for diagnosis, prevention, and management of a disease or for research purposes. Urinary pH should be determined by a pH meter in these scenarios (Chew and DiBartola, 1998; Defontis *et al.*, 2013; Kwong *et al.*, 2013; Athanasiou *et al.*, 2018). One of the problems with strips is that it can lead to variations in assessment between individuals since color variations may be subtle, and individual visual acuity for color perception can vary (Lanevschi-Pietersma, 2002; Raskin, Murray and Levy, 2002). However, the correlation observed in this study for both visual and automated methods in comparison with the pH meter is good by statistical criteria (>0.75). Nonetheless, in the present study only one person was reading the urinary pH strips which might give some bias.

Defontis *et al.* (2013) registered in companion animals, that automated reading of the dipsticks was better than visual reading and the better method for urinary dipstick examination. The likewise can be observed in the present study since the coefficient of correlation, with the pH meter, was 0.92 for the visual method and 0.97 for the automated method.

9. Conclusion

Results indicated that the oral supplement potassium citrate, and the therapeutic dry foods Hill's ® Prescription Diet® u/d® Canine (u/d) and Royal Canin Urinary S/O dog (Urinary S/O), can manipulate urinary pH. On the other hand, the solution containing ammonium chloride (Urical)

did not produce a significant urinary pH difference. It also showed at baseline and in all treatments, an increase in urinary pH between 9h00 and 11h00 (approximately 2-4 hours after food intake). Nonetheless, the specific circadian profile of urinary acidification remains imperfectly defined, and in order to formulate recommendations concerning feeding management, it is essential to know the effect of food intake on the 24-h day urinary pH.

Urinary pH estimation from the dietary analysis can be a valuable tool. However, it has to be kept in mind that there are a number of sources of inaccuracies and variations involved. There is not to the date a valuable method/equation that can aid in the estimation of urinary pH without a significant error, and so significant additional research, particularly in dogs, is required.

In the present study, it was confirmed that nutrition does influence acid-base balance in dogs, and it was evident that food can be a valuable tool to manipulate pH, establishing pH profiles for multiple ingredients/nutrients will serve in the development of diets designed to control urinary pH. Unfortunately, little published data exist evaluating dietary influence on urinary pH in dogs.

Appendix

Table 4: Nutrient composition of one maintenance adult dog diet and two therapeutic diets formulated to prevent recurrence of calculi in dogs.

Variable	Hill's science plan[a]	u/d[b]	Urinary SO[c]
Protein (g/100kcal)	5.8	2.5	4.7
Fat content (g/100kcal)	4.0	4.8	4.4
Carbohydrate (g/100kcal)	13.2	14.4	11.2
Crude fiber (g/100kcal)	0.48	0.6	0.59
Crude ash (g/100kcal)	1.29	0.8	1.7
Sodium (g/100kcal)	0.062	0.054	0.31
Calcium (g/100kcal)	0.196	0.104	0.13
Potassium (g/100kcal)	0.172	0.143	0.21
Phosphorus (g/100kcal)	0.172	0.042	0.13
Magnesium (g/100kcal)	0.027	0.013	0.013
Moisture (%)	8	7.5	9.5
Metabolisable energy (kcal/kg)	3718	3998	3866

a) Hill's science plan adult medium breed advanced fitness lamb and rice

b) Hill's™ prescription diet™ u/d™ canine, hill's pet nutrition.

c) Royal canin canine urinary so, royal canin.

Table 5: Ingredients of one maintenance adult dog diet, two therapeutic diets formulated to prevent recurrence of calculi in dogs, one ammonium chloride containing solution and potassium citrate.

Diet/supplement	Ingredients	Dosage
Hill's science plan[a]	Maize, wheat, lamb meal, soybean meal, animal fat, maize gluten meal, brewers' rice, hydrolyzed protein, vegetable oil, flaxseed, minerals.	
U/d[b]	Brewers Rice, Corn Starch, Pork Fat, Egg Product, Powdered Cellulose, Chicken Liver Flavor, Flaxseed, Lactic Acid, Potassium Citrate, Soybean Oil, Calcium Carbonate, L-Lysine, Iodized Salt, Choline Chloride, vitamins (Vitamin E Supplement, Niacin Supplement, Thiamine Mononitrate, Vitamin A Supplement, Calcium Pantothenate, Biotin, Vitamin B12 Supplement, Pyridoxine Hydrochloride, Riboflavin Supplement, Folic Acid , Vitamin D3 Supplement), Dried Beet Pulp, L-Threonine, Taurine, minerals (Ferrous Sulfate, Zinc Oxide, Manganous Oxide, Copper Sulfate, Calcium Iodate, Sodium Selenite), L-Carnitine, L-Tryptophan, Mixed Tocopherols for freshness, Natural Flavors, Beta-Carotene.	

Urinary so[c]	Brewers rice, corn, chicken fat, chicken by-product meal, brewers rice flour, corn gluten meal, natural flavors, salt, powdered cellulose, potassium chloride, vegetable oil, calcium sulfate, fish oil, monocalcium phosphate, DL-methionine, fructooligosaccharides, L-lysine, choline chloride, taurine, vitamins [DL-alpha tocopherol acetate (source of vitamin E), biotin, D-calcium pantothenate, vitamin A acetate, niacin supplement, pyridoxine hydrochloride (vitamin B6), thiamine mononitrate (vitamin B1), vitamin B12 supplement, riboflavin supplement, vitamin D3 supplement, folic acid], trace minerals [zinc proteinate, zinc oxide, ferrous sulfate, manganese proteinate, manganous oxide, copper sulfate, calcium iodate, sodium selenite, copper proteinate], marigold extract (Tagetes erecta L.), L-tryptophan, rosemary extract, preserved with mixed tocopherols and citric acid.	
Urical	Ammonium chloride, citric acid, iron chloride, glucose, potassium chloride, methionine, methylthionine, sodium chloride, flavouring agents, water, zinc sulphate.	5 mL/10kg BW/day
Potassium citrate	Potassium citrate	130-211 mg/kg BW/day

a) Hill's science plan adult medium breed advanced fitness lamb and rice

b) Hill's™ prescription diet™ u/d™ canine, hill's pet nutrition.

c) Royal Canin canine urinary SO, Royal Canin.

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