Department for Farm Animals and Veterinary Public Health

University of Veterinary Medicine Vienna

Institute of Animal Nutrition and Functional Plant Compounds

(Head: Univ.-Prof. Dr.sc.agr. Qendrim Zebeli)

Comparison of the buffering capacity of canine food in relation to manufacturing method and nutrient composition

Diploma Thesis

University of Veterinary Medicine Vienna

submitted by

Verena Stefani

Vienna, June 2023

Supervisor: Univ.-Prof. Dr.sc.agr. Qendrim Zebeli

Institute of Animal Nutrition and Functional Plant Compounds

Department for Farm Animals and Veterinary Public Health

University of Veterinary Medicine Vienna

Supervisor: Tzt. Annegret Lucke PhD.

Institute of Animal Nutrition and Functional Plant Compounds

Department for Farm Animals and Veterinary Public Health

University of Veterinary Medicine Vienna

Assessor: Priv. Doz. Dr. Franziska Roth-Walter

TABLE OF CONTENTS

1. Introduction

Buffering capacity (BC) is defined as the resistance of a solution to change pH with the addition of an acid or a base (Van Slyke 1922). In the context to gastric digestion the buffering capacity of food affects the absorption of hydrogen ions and therefore the gastric juice pH (Levic et al. 2005). The gastric juice pH is in turn a key regulatory parameter of digestion, as the gastric milieu pH plays an important role for the activity of gastric enzymes, solubility of dietary ingredients, and therefore in the absorption of nutrients across the gastrointestinal tract (Smeets-Peeters et al. 1998). Furthermore, the gastric pH acts as a barrier against foodborne pathogens (Zentek 2022), and recently a study found that the gastric pH has also an influence on the gastrointestinal microbiota of dogs (Garcia-Mazcorro et al. 2012). If the gastric pH remains too high after food intake, it could lead to a decreased digestion and an increased risk of an infection with foodborne pathogens (Zentek 2022).

The chemical and physical properties of the food itself are of high importance in the context of gastric digestion. Indeed, the gastric breakdown of food nutrients is directly influenced by food properties, so that besides the physicochemical composition, the BC is also an important parameter. Because of this, measuring the BC of feedstuffs is common in ruminant, pig, and poultry dietary ingredients (Jasaitis et al. 1987, Giger-Reverdin et al. 2002, Lawlor et al. 2005, Levic et al. 2005, Montañez-Valdez et al. 2013). Results of these studies have shown that meat and fish meal, milk products, amino acids, root and pulps products and vegetable proteins possess high BC (Lawlor et al. 2005). It seems that feedstuffs with a high protein content possess an inherent high BC (Lawlor et al. 2005, Levic et al. 2005, Montañez-Valdez et al. 2013), thus, the protein content raise the BC of a feed/food ingredient. Besides, the ash content of foods seems also to be an important factor influencing the BC of feedstuffs (Jasaitis et al. 1987).

The BC of food and ingredients used for human consumption has also been frequently in the focus of research in the context of gastric digestion (Salaün et al. 2005, Al-Dabbas et al. 2010, Mennah-Govela et al. 2020, Ebert et al. 2021). For example, Salaün et al. (2005) analysed the BC of dairy products and found out that the BC depends on the composition of minerals and proteins. Al-Dabbas et al. (2010) measured the BC of legumes, almond, lettuce stem, carob,

liquorice root and raw cow milk. They found a strong positive correlation of BC with protein, aspartic and glutamic amino acids contents. Mennah-Govela et al. (2020) measured the BC of thirty commercially available foods. They identified protein content and initial pH as the most important factors in determination of BC. Also, interestingly, the particle size of the food seemed to be an important factor, but it was influenced by the protein content, too (Mennah-Govela et al. 2020). Ebert et al. (2021) detected ash and selected minerals and amino acids, with a pka in the range of foodstuff e. g. aspartic and glutamic acid, as key influencing factors on overall BC, by analysing the BC of wet texturized plant proteins in comparison to pork meat, with a focus on the compositional differences.

Until now there are no studies dealing with the BC of dog foods or dietary ingredients. This is surprising taking into account the importance of the BC not only for the canine gastric digestion but also the dog health such as gastric hypoacidity or dilation-volvulus syndrome (Zentek 2022), food allergenicity (Pali-Schöll et al. 2018), and dental health (Hale 2009). In addition, the design of oral canine drugs and pharmaceutical requires knowledge of the food BC, too. It had also to be mentioned that the dog diet is very complex and extends from inclusion of meat, meat products, inner organs and bones to dairy, eggs, and marine food products, and further to plant-based ingredients such as grains, legumes, seeds, and vegetables, as well as mineral and vitamin and feed additive supplements. These dietary ingredients are commonly fed as commercially available complete food, either as dry kibbles or wet food, but also as homemade diet. Therefore, both the ingredients and the manufacturing method may affect the BC of the dog food. Knowledge of the BC and pH of canine food may help prediction of the effects of the diet on digestion and health in dog. The aim of this study is to analyze and compare the BC of commercial dry and wet dog foods as well as homemade dog food in relation to their manufacturing method and ingredients.

In consideration of the findings of previous studies in other animal species and humans, we hypothesized that the protein content of the dog food has a major influence on its buffering capacity.

2. Literature review

2.1 Physiology of the canine stomach and duodenum

2.1.1 Anatomy and main roles of the stomach in dogs

The stomach of the dog is simple and fully covered with glandular mucosa. There are three different types of gastric mucosa. The cardiac mucosa is located annular at the stomach entrance and the glands produce mucus to buffer the gastric juice. The gastric mucosa is located at the body and the fundus of the stomach and there are parietal cells that produce hydrochloric acid that acidifies the juice and chief cells that produce pepsinogen. The pyloric mucosa covers a part of the body, the pyloric antrum, and the pyloric canal. The glands produce an alkaline mucus and a small amount of pepsinogen (Salomon et al. 2020). Lipase is found to be secreted by mucous neck cells and mucous pit cells of gastric glands of the cardia, corpus, and pyloric antrum (Steiner et al. 2002).

The stomach has three main functions. First, it serves as a storage area for food, which in dogs allows the intake of bigger meals. Second, the secretion of enzymes to start the digestion of proteins and triglycerides. Third, the gastric motility ensure the adequate grinding of the food and regulate the transport of the chyme to the duodenum (Breves et al. 2022).

2.1.2 Gastric acid secretion

Dogs have with 0.1 mEq h^{-1} compared to humans with 2-5 mEq h^{-1} a very low basal gastric acid secretion rate, and this may explain differences in the basal gastric pH, which is lower in humans (Kararli 1995). However, after stimulation the gastric acid secretion is in dogs with 39 mEq h^{-1} higher than in humans with 18-23 mEq h^{-1} (Kararli 1995). Many factors like volume/amount and the composition of food influence the gastric acid secretion (Smeets-Peeters et al. 1998). It has been shown that the amount of protein in a meal is the most important stimulant for acid secretion (Brooks 1985), because amino acids and digested protein induce

gastrin release (Walsh 1988). It is gastrin that stimulates the parietal cells of the stomach to produce the hydrochloric acid (HCl). In addition, the release of gastrin is also stimulated by the vagus nerve. The decrease of the gastric pH induce the release of somatostatin, which leads to a paracrine inhibition of gastric acid secretion (Breves et al. 2022).

2.1.3 Gastric pH regulation and its role for digestion and foodborne pathogens

The gastric pH is a parameter that reflect the balance between the gastric acid secretion, buffering agents in the stomach as well as those of the food. The gastric pH in fasted dog varies very widely, and there are conflicting data in the literature regarding dog gastric pH, which can also be explained by different methods used to measure the pH, as well as the intragastral location. Therefore, the mean pH in fasted dogs in different studies ranged from 2 to 7 (Banta et al. 1979, Akimoto et al. 2000, Sagawa et al. 2009, Mahar et al. 2012).

In fed dogs the gastric pH varies, depending on literature sources, from 1.08 to 2.4 (Banta et al. 1979, Sagawa et al. 2009). In one study of dogs were fed either with dry or canned food, after eight hours the average pH was 2.4 regardless of the diet (Banta et al. 1979). Another study found out that the gastric pH in dogs fed with 10 g dry food was significantly lower than in dogs fed with 200 g dry food, suggesting that the amount of food serves as a buffering agent. Another reported also that the amount of food consumed has a significant influence on the gastric pH. For example, the pH of dogs fed with 10 g of dry food was 1.08 compared to 1.26 of dogs fed with 200 g of dry food. Indeed, in 200 g fed dogs the gastric pH with 2.11 was significantly higher in the first 60 minutes (Sagawa et al. 2009). Mahar et al. measured pH peaks in the first 60 minutes after a meal with a interquartile range from 5.3 to 5.7 fed in the home cage or 3.5 to 4.5 fed in the study cage. (Mahar et al. 2012), likely the surroundings also played a role in the gastric acid secretion.

The gastric pH plays an important role for digestion, because the pH regulates the activity of digestive enzymes (Smeets-Peeters et al. 1998), which are described in more details in the next chapter.

The acidity of the stomach is also important barrier against foodborne pathogens (food and water). The bacteriostatic effect is against bacteria with a pH optimum between 4 and 6 (Zentek 2022).

Indeed, gastric acid secretion is a regulator for the bacterial colonization in the small intestine and a decreased acid output can be associated with intestinal dysbiosis (Suchodolski 2016). A study, where dogs received a gastric suppressant therapy with a proton pump inhibitor, showed an quantitative change in the gastrointestinal microbiota (Garcia-Mazcorro et al. 2012). These findings suggest that the gastric pH can have an influence on the gastrointestinal microbiota of dogs, and hence the gastrointestinal health.

2.1.4 Enzymes in the gastric juice and importance of pH for their activity

Pepsin and lipase are the major digestive enzymes present in the gastric juice of dogs (Smeets-Peeters et al. 1998).

Canine gastric lipase is secreted by mucous pit cells of gastric glands (Carrière et al. 1992). Gastric lipase initiates the fat digestion in the stomach and splits triacylglycerols mostly in diacylglycerol and free fatty acids (Breves et al. 2022). The activity of gastric lipase is pH dependent, so that pH optimum of gastric lipase is between pH 3 to 7 (Breves et al. 2022). At pH 4 the gastric lipase is 13 time more active on long-chain than on short-chain triacylglycerols, medium-chain triacylglycerols has its pH optimum at 6 (Carrière et al. 1991). Gastric lipase is irreversibly inactivated below pH 1.5 (Carrière et al. 1992), so that buffering the acids in the stomach is important to prevent this lipase inactivation.

Pepsinogen is secreted by chief cells and hydrochloric acid in the lumen of the stomach activates pepsinogen to pepsin. Pepsin splits peptide bonds from protein with aromatic amino acids (Breves et al. 2022). The optimum pH for pepsin is 2 and it gets inactivated at neutral pH (Smeets-Peeters et al. 1998). When dogs ingest collagen-rich food, pepsin is most active, because of that its activity is more important for initiating the digestion of meat than of vegetable protein (Maskell et Johnson 1993).

2.1.5 Gastric emptying

Gastric emptying is defined as the transport of food to the duodenum in a rate and form that optimize the intestinal absorption of nutrients (Wyse et al. 2003). The gastric emptying rate is affected by many parameters like volume, energy density, viscosity, density and particle size of the food, temperature, body weight and amount of acids in the duodenum (Smeets-Peeters et al. 1998).

There is a difference in the emptying mechanism of solid and liquid food. Solid food has to be broken down to a small particle size with a diameter less than 2 mm in order to escape the stomach (Hinder und Kelly 1977). For the escape, the food is first mixed to a semiliquid chyme and the chyme is moved towards the pylorus. On to the way towards the pylorus the peristalsis increases in amplitude and velocity. The pylorus and the proximal antrum close, when the wave of contraction reaches the distal antrum and particles, which are too big to pass the pylorus, were thrown back into the body of the stomach. This process is repeated until the particles are triturated enough to leave the stomach (Wyse et al. 2003). Liquid food empties in a monoexponential manner and is determined through the pressure gradient between stomach and duodenum (Smeets-Peeters et al. 1998). Whereas solid food empties in a linear pattern after a lag phase and is retained in the fundus until most of the liquid has left the stomach (Horowitz et al. 1994).

Indigestible particles, that are smaller than 1,6 mm in diameter are transported directly in the duodenum, whereas bigger particles, that cannot be milled, are retained in the stomach until the digestible solids have emptied (Wyse et al. 2003). Those too big particles are emptied during interdigestive periods (Horowitz et al. 1994).

The gastric emptying time varies among different studies (Smeets-Peeters et al. 1998). A study using external scintigraphy and feeding the dog 255 g of canned dog food measured a mean gastric half-emptying time of 77 min (Theodorakis 1980). Another study using scintigraphy, that was feeding dry kibble food to the dog, found a mean gastric half-emptying time of 240 min (Hornof et al. 1989).

2.1.6 Duodenal pH

The mean pH of the duodenal bulb in fasted dog is 5.0±0.6 and postprandial the lowest mean pH is 3.4±0.3, which occurs between 15 min and 30 min after feeding (Brooks und Grossman 1970a). When the pH in the duodenum drops below pH 4.5, secretin is released (Meyer et al. 1970). Secretin inhibits gastric motility, gastrin-stimulated acid secretion and stimulates pancreatic, hepatic and Brunner gland bicarbonate output (Brooks und Grossman 1970b). Bicarbonate buffers the chyme to increase the pH, which is needed to activate the pancreatic enzymes (Banta et al. 1979).

A study from Itoh et al. measured the daily changes of duodenal pH for 24 hours. They divided the pH changes into three periods. The weak acid period has pH a pH range between 7 and 4 and a mean duration time from 11.4±0.91 h after meal. Then follows the strong acid period with a pH range between 8 and 1 and a duration of 5.1±0.63 h after the weak acid periods. The alkaline period begins 16.5±0.77 h after feed and has a mean pH from 7.8±0.21 (Itoh et al. 1980).

2.1.7 Pancreatic juice

The high level of bicarbonate in the pancreatic juice neutralizes the acid chyme from the stomach, to create the optimal conditions for the pancreatic enzymes to work. The pancreatic juice contains important enzymes for the digestion of protein, fat and carbohydrate (Zentek 2022).

The secretion of the pancreatic juice is during the digestive phase after feeding. The amount and type of food also play a role in the composition and secretion rate of pancreatic juice (Smeets-Peeters et al. 1998).

The enzymes (trypsin, chymotrypsin, carboxypeptidase, elastase) for the digestion of protein are inactivated. Trypsin gets activated in the intestinal lumen by enterokinase, which is secret

by enterocytes of the duodenum and activates the other enzymes. This mechanism acts as protection from self-digestion (Zentek 2022).

The pancreatic lipase hydrolyses triglycerides in di- and monoglycerides and fatty acids (Zentek 2022).

Dogs have a high amylase activity that increases with starchy feeding (Kienzle 1988). Amylase breaks down polysaccharides to disaccharides (Kues et al. 2021).

2.1.8 Bile

The liver produces bile, which is stored in the gall bladder between meals (Smeets-Peeters et al. 1998). Between 29 and 53 per cent of the newly produced bile is stored in the gall bladder and the rest in released directly into the duodenum (Rothuizen et al. 1990). The water of the bile gets resorbed in the gall bladder, so that the bile is more concentrated compared to the bile directly secreted from the liver (Breves et al. 2022). Bile acid promotes the digestion of fat, it activates the pancreatic lipases and transfers breakdown products from fat in a hydro soluble form (Zentek 2022). Food ingestion induces the emptying of the gall bladder. Breakdown products of the protein and fat digestion causes the release of cholecystokinin, that initiate the contraction of the smooth muscles of the gall bladder (Breves et al. 2022). Gallbladder emptying is the most rapid during the first 30 min after feeding and is completed after 2 h (Traynor et al. 1984).

2.2 Buffering capacity of the food

The BC is a physicochemical characteristic of food and is strongly affected by the presence of acid/base groups, which causes a resistance to change pH after additions of acid or akali (Van Slyke 1922). The BC of foods is an important parameter with a crucial role for the enzymatic breakdown; yet, this has been largely disregarded in dogs as compared with humans and farm animals. A study that measured the buffering capacity of pig diet ingredients identified

minerals, acid salts, meat fish meal, milk products, amino acids and vegetable proteins as ingredients with a high buffering capacity (Lawlor et al. 2005). In human studies, the protein content has been identified as the most important factor in determination of BC of food (Mennah-Govela et al. 2020). Feedstuffs with a crude protein (CP) content of more than 15% have a high buffering capacity (Montañez-Valdez et al. 2013). BC of proteins comes from the ionizable groups on the polypeptide chains, including the side chains of the amino acids, the terminal α-amino groups and the terminal α-carboxyl groups (Luo et al. 2018). Studies have shown that the concentrations of glutamic and aspartic acid, have an important impact on BC, too (Luo et al. 2018, Mennah-Govela et al. 2019). Organic acids also play a role in buffering capacity of food. In food with a low protein content organic acid as lactic acid, malic acid, acetic acid, phosphoric acid, and citric acid acts as buffer (Mennah-Govela et al. 2020). It has been shown that carrot juice has compared to other juices a high buffering capacity. The authors of the study assumed that high malic acid and glutamic acid content in carrot juice cause the high buffering capacity (Mennah-Govela et al. 2020). Beside the food composition, the particle size and physical state of food has been shown to influence the buffering capacity of food (Mennah-Govela et Bornhorst 2021). A study suggested that particle size impacts buffering capacity by limiting acid diffusion into food particles, therefore larger particle have a lower buffering capacity compared to smaller ones (Mennah-Govela et al. 2020).

2.2.1 Dietary cation anion balance

The dietary cation anion balance (DCAB) describes the ration from cations and anion in feedstuff and has an influence on the acid-base balance (Rérat et al. 2010). For the calculation of the DCAB strong ions are used, they are fully dissociated at physiologic pH and have no buffer function, but they influence the acid-base balance directly (Constable 1999).

In dogs, the DCAB is used to predict and modify the urinary pH for urolith prevention and treatment (Yamka et Mickelsen 2006, Rückert 2015). An increased intake of anion leads to a decrease in urine pH, whereas an increased intake of cations leads to an increase of urine pH (Rückert 2015).

2.3 Pathologies that might be influenced by the buffering capacity of food

2.3.1 Saliva pH and dental caries

Bacterial decay of the tooth structure caused by release from acids from oral bacteria fermenting carbohydrates on the tooth surface is the reason for caries (Hale 2009). The incidence of caries with 5.25 % in adult dogs, is lower than in humans (Hale 2009). Reasons for the low incidence of caries in dogs are conical tooth shape, wider inter-dental spacing, diets with less carbohydrate and higher salivary pH to buffer acids compared to humans (Hale 2009). A study reported a mean pH of 7.93±0.46 for saliva of dogs fed with commercial dry dog food (Iacopetti et al. 2017). Yet, the pH of the saliva can be influenced by food (Smeets-Peeters et al. 1998), and this context the initial pH of the food might need a closer consideration. It is possible that for example a low pH of dog food can lead to a higher risk of caries, because it lowers the pH of the saliva, but further research is needed.

2.3.2 Gastric hypoacidity

Dogs with gastric hypoacidity have an insufficient production of hydrochloric acid in the stomach and are often showing vomiting (Zentek 2022). The cause of this disease can be organic, psychic or due to a lack of chloride (Zentek 2022). Hypoacidity is also a clinical sign of chronic atrophic gastritis (Patel et al. 2018).

The protein digestion is hindered due to the lack of hydrochloric acid, as the pH in the stomach is too high and the effect of pepsin is decreased, and the gastric emptying is accelerated. This can also lead to diarrhoea, because the proteins cannot be properly be split up in the small intestine (Zentek 2022). In this context the BC of food could be helpful to choose the right diet. For patients with gastric hypoacidity a diet with a low BC is needed to keep the amount of gastric acid needed, to reach an optimal gastric pH of 2, low.

2.3.3 Gastritis

Gastritis is the inflammation of the gastric mucosa and can be acute or chronic. Dogs with acute gastritis show sudden onset of vomiting, whereas dogs with chronic gastritis show intermittent vomiting over 1-2 weeks (Patel et al. 2018).

The stimulation of the gastric wall by foreign materials, chemical injury, ischemia, infection, or antigens leads to the release of inflammatory components, which stimulate acid secretion. This leads to mucosal breakage and through increase of the permeability of the epithelial layer and altered blood flow to a higher risk of gastritis, gastric erosion and ulceration (Patel et al. 2018).

Food that leads to an excessive gastric acid production must be avoided. The meal should contain a minimum of protein because it increases the acid secretion (Zentek 2022). In this chase food with a low BC is needed to avoid further acid production.

2.3.4 Gastric dilatation-volvulus syndrome

The gastric dilatation-volvulus syndrome is a potential life-threatening disease that mainly occurs in large, deep-chested dogs. The dilatation of the stomach due to rapid ingestion of large meals or accumulation of gas, can lead to a volvulus. Until now the exact cause for the pathogenesis is still unknown, only risk factors were identified (Steiner und Allenspach 2011).

A possible risk factor is food with a high content of crude ash. Ash can buffer the gastric content which leads to an insufficient pH drop in the stomach. A high pH in the stomach promotes the growth of gas producing bacteria (Zentek 2022). An accumulation of gas increases the risk of a gastric dilatation-volvulus syndrome.

2.4 Aim and hypothesis

The aim of the study was to find out whether there is a difference in the buffering capacity of different dog foods in order to draw conclusions about which composition and production method has a beneficial influence on the digestion process. We hypothesized that the composition and production method of dog food influenced the BC, and we assumed that dog food with a high protein content had higher BC than dog food with a low protein content.

3. Materials and methods

3.1 Dog foods used in the experiment

In this experiment, a total of 30 complete dog foods were investigated. In detail, each ten different types of commercial dry and canned dog food and homemade dog food were used. The analytical composition as declared on the food label of the dry and wet feed are shown in Table 1 and the ingredients of the commercial dog food is shown in Table 2. The ingredients of the homemade dog food are shown in Table 3.

Tab. 1: Analytical constituents of the commercial dry and wet dog food (as declared by the manufacturers)

ID	Crude protein [%]	Crude fat [%]	Crude fibre [%]	Crude ash [%]	Moisture [%]
Wet food 1	9.8	5.5	0.3	1.6	81.0
Wet food 2	11.0	6.0	0.5	1.6	74.0
Wet food 3	8.5	4.0	0.5	2.0	82.8
Wet food 4	10.0	5.0	0.3	2.2	82,0
Wet food 5	10.5	5.6	0.3	2.4	80,0
Wet food 6	14.5	9.0	0.6	2.5	69.0
Wet food 7	11.0	6.5	0.4	2.0	78.0
Wet food 8	10.5	8.0	0.5	2.5	75.0
Wet food 9	11.0	7.0	0.6	2.5	78.0
Wet food 10	7.7	5.5	0.5	2.5	81.1
Dry food 1	27.0	16.0	1.4	5.4	undeclared
Dry food 2	21.0	12.0	2.5	7.3	20.0
Dry food 3	21.0	10.0	3.0	8.0	undeclared
Dry food 4	23.0	11.0	3.5	7.0	undeclared
Dry food 5	23.8	12.2	2.2	6.8	9.0
Dry food 6	23.0	10.5	3.4	7.0	10.0
Dry food 7	21.0	13.0	1.5	7.5	undeclared
Dry food 8	21.5	10.5	2.5	5.9	8.0
Dry food 9	26.0	16.0	2.0	7.9	19.0
Dry food 10	22.0	9.0	3.0	6.5	undeclared

Tab. 2: Ingredients of dry and wet dog food (as declared by the manufacturers)

ID	Ingredients
Wet food 1	Meat and animal derivatives (including 4% derivatives from beef), minerals, oils and fats, derivatives of vegetable origin, yeast (0,18%)
Wet food 2	Meat and animal by-products (lamb and chicken), vegetables (potato), minerals
Wet food 3	Meat and animal derivatives (50%, including 4% poultry), vegetables (including 4% mix of carrots and peas), grain (4% cooked rice), minerals, derivatives of vegetable origin
Wet food 4	57% meat and animal derivatives (including 30% from chicken and 4% from turkey), 4% fish and fish derivatives, grain (4% brown rice), minerals, oils and fats (0,8% salmon oil)
Wet food 5	Beef (21%), turkey, pork, dehydrated pork proteins, minerals, carrots (0,9% dehydrated carrots, corresponds to 8,1% carrots)
Wet food 6	Fresh turkey meat (42%), fresh pork meat (35%, Iberian pork only), fresh chicken meat (6%), salmon oil, dehydrated potato, dehydrated vegetables (broccoli, carrots, leek), dried brewers' yeast, olive oil
Wet food 7	32% salmon, 30% chicken (heart, liver, gizzard), stock form salmon, 29% chicken, 4% zucchini, 2% amaranth, 1% chia seeds, 1% krill, 0,5% minerals, 0,5 dried eggshells, 0,5% salmon oil
Wet food 8	68% lamb (heart, lung, liver, rumen), 2% amaranth, 0,8% cranberries, 0,2% salmon oil, calcium carbonate, sodium chloride
Wet food 9	Meat and animal derivatives (among other 10% venison), bakery products (5% cooked pasta), minerals, oils and fats (0,2% flaxseed oil), derivatives of vegetable origin (0,1% inulin)
Wet food 10	Meat and animal derivatives (51% including 21% beef, lamb and chicken), grain, minerals, derivatives of vegetable origin (among other 0,5% dried beet slices), oils and fats (among other 0,5% sunflower oil)
Dry food 1	Dehydrated poultry protein, maize, maize flour, animal fats, maize gluten, vegetable protein isolate, wheat, hydrolysed animal proteins, rice, beet pulp, minerals, fish oil, soya oil, yeasts and parts thereof, fructo-oligosaccharides
Dry food 2	Grain, meat and animal derivatives (4% from beef), derivatives of vegetables origin, oils and fats, sugar, vegetables (4% carrots), minerals
Dry food 3	Grain (wholemeal 57%), meat and animal derivatives (15%), derivatives of vegetable origin, oils and fats, vegetable protein

	extracts, glycerin, minerals, propylene glycol, dehydrated vegetables (0,25% peas and 0,25% carrots)
Dry food 4	Meat and animal derivatives 28% (4% veal), rice, maize, barley, millet, beef tallow, carrots 5%, chicory 2%, barm 1%, lignocellulose, flaxseed, blueberries 0,5%, seaweed, sodium chloride, dried dandelion, dried field horsetail, dried comfrey, dried borage, dried echinacea, dried rampion
Dry food 5	Cereals (maize, rice), meat and animal by-products (poultry meat meal, lamb meat meal), oils and fats, vegetable by-products (slivers of beet molasses), minerals, eggs and egg products (whole egg powder), yeast, algae (Ascophyllum nodosum), seeds (linseed), herbs, yeast extract (source of MOS), green-lipped mussel (Perna canaliculus), dried
Dry food 6	30% poultry meat, 16% maize, dried poultry protein, rice, 14% potato flour, 3% fishmeal, beet pulp, carob meal, dried barm, hydrolysed poultry liver, poultry fat, vegetable oil, dicalcium phosphate, sodium chloride, potassium chloride, dried herbage, Yucca schidigera
Dry food 7	Grain, meat and animal derivative (14% including 4% chicken), oils and fats (among other 0,4% sunflower oil), vegetable protein extracts, derivatives of vegetable origin, minerals, vegetables
Dry food 8	Fresh poultry (30 %), millet (20 %), maize, poultry protein (dried), dried beet pulp (sugar removed), rice, poultry fat, hydrolysed protein, linseed, powdered egg, fish meal, fish oil, peas (dried), yeast (dried, 0.1 % mannanoligo saccharides, 0.06 % beta-glucans), sodium chloride, green-lipped mussels (dried, 0.1 %), potassium chloride, chicory (dried)
Dry food 9	Fresh chicken meat (70 %), broken rice, corn (GMO-free), dehydrated chicken protein, hydrolysed poultry protein, dried brewers' yeast, dried apple pulp, salmon oil, yucca extract, cold-pressed flaxseed oil, cold-pressed olive oil, green-lipped mussel extract, dried carrots, dried tomatoes, dried african marigold, dried dandelion, dried broccoli, dried green tea, dried chamomile, dried oregano, dried milk thistle seed, dried cranberry seed, dried seaweed, potassium chloride
Dry food 10	Poultry protein, whole wheat, whole-grain maize, wheat flour, maize flour, whole-grain barley, lamb protein (8%), rice flour (8%), beet pulp (desugared), poultry fat, beef fat, hydrolysed liver, fish meal, sunflower oil (0.8%), apple pomace (0.8%), yeast, potassium chloride, rapeseed oil (0.2%), sodium chloride, green oats, sunflower, cress, parsley, green herbs (total: 0.3%) dried

Tab. 3: Ingredients of homemade dog food

ID	Ingredients
Homemade 1	60.3% potato, 36.2% codfish, 2.4% rapeseed oil, 1% vitaminized mineral feed, 0.1% sodium chloride
Homemade 2	52.6% green rumen, 44% potato, 2.1% rapeseed oil, 1.2% vitaminized mineral feed, 0.1% sodium chloride
Homemade 3	63.5% rice, 28.4% chicken, 5.4% carrots, 1.3% rapeseed oil, 1.3% vitaminized mineral feed, 0.1% sodium chloride
Homemade 4	59.3 rice, 21.2% chicken, 12.1% curd, 4.9% carrots, 1.2% rapeseed oil, 1.2% vitaminized mineral feed, 0.1% sodium chloride
Homemade 5	42.4% rice, 25.2% horse meat, 17.5% boiled egg, 12.1% carrots, 1.5% vitaminized mineral feed, 1.2% rapeseed oil, 0.1% sodium chloride
Homemade 6	43.4% rice, 41.3% horse meat, 12.4% carrots, 1.5% vitaminized mineral feed, 1.3% rapeseed oil, 0.1% sodium chloride
Homemade 7	53.1% oat flakes, 36.5% head meat from beef, 7.6% carrots, 1.5% vitaminized mineral food, 1.1% rapeseed oil, 0.2% sodium chloride
Homemade 8	56.2 oat flakes, 34.9% muscle meat from beef, 6.4% carrots 1.3% vitaminized mineral feed, 1.3% rapeseed oil, 0.2% sodium chloride
Homemade 9	45.2% pasta, 42% beef lung and heart, 10% carrots, 1.3% rapeseed oil, 1.3% vitaminized mineral feed, 0.2% sodium chloride
Homemade 10	61.5% pasta, 25.6% beef lung and heart, 10.3% carrots, 1.4% vitaminized mineral feed, 1% rapeseed oil, 0.2% sodium chloride

The commercial dog feeds were purchased from different local supermarkets and pet stores to provide variability of products with different producers and compositions. For compliance with nutritional standards, the producers of the commercial complete dog feeds had to be member of the “Industrieverband Heimtier (IHV) e.V.” or the “Österreichische Heimtierfuttermittel Vereinigung (ÖHTV)”. Membership in one of these associations obliges the companies to produce their dog food according to the FEDIAF Nutritional Guidelines, they provide for example information about the recommended nutrient levels for dog food according to the current scientific research.

The homemade dog foods were calculated to provide enough energy and nutrients for an adult, inactive dog using an Excel® calculation program (“CarnivoreDiet” ©, A. Lucke, Vetmeduni Vienna, Austria) according to Nutrient requirements of cats and dogs (National Research Council 2006). The meat used for the homemade diets was bought frozen and already minced in local pet stores. The vegetables were bought fresh in a local supermarket. On the day of analysis, the meat was cooked. Only the green rumen was used raw in the diet. The amount of used ingredients was calculated for a dog with 7 kg and were weighed with a scale (ME4002®, METTLER TOLEDO, USA) before mixing them together.

3.2 Sample preparation

For the sample preparation 150 g of commercial dog food or the entire homemade dog food was mixed in a knife mill (Grindomix® GM 200, Retsch, Germany). Canned dog food was homogenized five times for each ten seconds at a speed with 5000 rotations per minute (rpm), dry dog food ten times for each ten seconds with 5000 rpm, homemade dog food three times for each 30 seconds with 5000 rpm. Different mixing times were necessary to obtain comparable texture of the different dog feed types. The texture of the dog food was not measured, the right texture was decided after visual judgement.

Afterwards 5 g of homogenized food and 15 g of distilled water (B30, adrona, Latvia) were weighed (ME4002®, METTLER TOLEDO, USA) and mixed in a 100 ml beaker. The final weight of each sample was 20 g.

The rest of the homogenized food and an aliquot of at least 100 g unhomogenized dry and wet dog food was frozen for further analyses. Homemade dog food was frozen only homogenized.

For the pH measurements a 0.16 M HCl was needed. For preparation of the 0.16 M HCl a 0.1 mol/l ampoule for preparation of Volumetric Solutions (ROTI®VOLUM, Carl Roth GmbH + Co. KG, Germany) was used. Overall, a total volume of 625 ml distilled water was added to a 0.1 ml/l ampoule of HCl to get the 0.16 M hydrochloric solution.

3.3 pH measurement

The measurement of the buffering capacity was done by acid titration method based on a previous publication (Mennah-Govela et al. 2020).

The pH-measurements were done with a portable pH meter with a DHS electrode (pH 7®, Xs-Instruments, Italy). The electrode was calibrated at room temperature using standard buffer solution (Technical Buffer Solution, Mettler Toledo, Switzerland) and had to reach an accuracy from 95 to 105 % before measuring the pH.

At first, the pH value of the undiluted wet and homemade dog food was measured.

The feed samples were stirred with a magnetic stirrer (MR Hei- Standard®, Heidolph Instruments, Germany) a total of 5 minutes with 250 rpm. Afterwards the initial pH of each sample was measured.

Then 0.5 ml of 0.16 Mole HCl were added, and the samples were stirred for 30 seconds.

After each addition of HCl the pH of the sample was measured.

Once a total of 7 ml HCl was added to the sample, 1 ml of HCl was added instead of 0.5 ml. As soon as a total of 30 ml HCl was added, 2 ml HCl were added to the sample instead of 1 ml.

This procedure was repeated until the sample reached a pH value < 2.0.

For the quality control, a duplicate sample was measured for each dog food and each pH measurement was performed in triplicate.

3.4 Proximate chemical (Weender) analyses

All food samples were analysed for dry matter (DM), ash, crude protein (CP), ether extracts (EE), acid detergent fibre (ADF) and neutral detergent fibre (NDF) according to the VDLUFA methods. The non-fiber carbohydrates (NFC) were calculated (NFC=100-(NDF+CP+EE+ash). The DM content of the samples was analysed by oven-drying at 103°C overnight, wet and homemade dog food had to be freeze-dried before oven-drying. Ash was analysed by

combustion of samples overnight by 580°C. CP was analysed by the Kjeldahl method. EE was analysed as ether extract using Soxhlet extractor (Extraction Sys-tem B-811; Büchi, Flawil, Switzerland). ADF and NDF were analysed using the Fiber Therm FT 12 (Gerhardt GmbH & Co. KG, Königswinter, Germany).

For further calculations the results of the Weender analyses were used.

3.5 Calculations

All calculations were done with Excel (Microsoft Excel, Microsoft Corporation®, United States of America).

First, the average of each triplicate pH measurement was calculated, this was also done with the duplicate of each sample. For further calculations the average of each sample and its duplicate was used.

The calculation of the buffering capacity was based on acid titration curves (Mennah-Govela et al. 2019).

$$Total\ buffering\ capacity = total\ acid\ added//\Delta pH$$

$$\Delta pH = initial\ pH - final\ pH$$

Based on the total buffering capacity, the buffering capacity per gram dry matter of the dog food was calculated. Also, the HCl use per g dry matter was calculated based on the total HCl use.

3.6 Statistical Analysis

For the statistical analysis the SAS (Version 9.4, SAS institute, Cary, NC, USA) was used. First the data was proved for normality. Afterwards an analysis of variance (ANOVA) using MIXED procedure of SAS was performed. The factor food type was defined as fixed effect and the

independent food samples as random effect. The Kenward-Roger method was used to approximate the degrees of freedom. A Tukey adjustment was applied to compare the means.

A multiple regression analysis was performed with the backward elimination procedure to evaluate the influence of different dietary effects on HCl use per g DM and BC per g DM with PROC REG of SAS. The variance inflation factor (VIF) was used to prevent correlations among the predictors. The fitness of the model was tested using R^2 and the root mean square error (root MSE).

Graphs of linear regression between different parameters were generated with Excel (Microsoft Excel, Microsoft Corporation®, USA).

The p values with < 0.05 were considered as significant and p values < 0.01 as highly significant.

4 Results

4.1.1 Comparison of declared vs measured nutrients in dry and wet dog food

In Table 4 the deviation of the measured vs. declared nutrients from manufactures in wet and dry dog food are shown.

The greatest deviation was found in EE content of wet food, in average the measured EE content was 13.3 % lower than declared by the manufactures. In wet food 1, 4, 5 and 7 the measured EE content was > 10 % lower than declared. In wet food 3 and 10 the measured EE content was > 30 % lower than declared. The ash content in wet food was in average 8.3 % higher than declared. In wet food 1and 2 the ash content was > 10 % higher than declared. In wet food 9 the ash content was > 20 % higher than declared and in wet food 6 > 60 % higher than declared. The ash content was >10 % lower in wet food 7 and in wet food 10 > 20 % lower than declared. The measured CP in wet food was in average 4.8 % lower than declared. In wet food 1, 2, 7 and 8 the CP was > 10 % lower than declared. Wet food 5 had a CP content > 10 % higher than declared. The measured DM content in wet food was in average 4.2 % higher than declared. In wet food 5, 6, 7 and 9 the DM was > 10 % higher than declared. Wet food 4 had a DM content > 20 % higher than declared. Wet food 2 had a DM content > 10 % lower than declared.

Also, in dry food the greatest deviation was found in EE content, in average it was 7.9 % lower than measured. In dry food 7 and 8 the EE content was > 20 % lower and in dry food 6 > 30 % lower than measured. Unlike wet food, in dry food the ash content was measured in average 5.6 % lower than declared. In dry food 4, 5, 6, 7 and 8 the ash content was > 10 % lower than declared. Dry food 10 had an ash content > 10 % higher than declared. The measured CP content in dry food was in average 3.3 % higher than declared. In contrast, dry food 1 had a measured CP content > 10 % lower than declared. The DM content of dry food was in average 2.9 % higher than declared.

Tab. 4: Deviation of the measured vs. declared nutrients from manufactures in wet and dry dog food [1]

ID	DM	CP	EE	Ash
Wet food 1	-3.5%	-18.9%	-19.8%	19.1%
Wet food 2	-18.7%	-10.3%	6.7%	10.7%
Wet food 3	-0.7%	-1.8%	-34.7%	-6.8%
Wet food 4	21.6%	1.1%	-18.4%	8.0%
Wet food 5	12.4%	13.2%	-24.4%	1.3%
Wet food 6	11.4%	0.6%	0.1%	63.9%
Wet food 7	19.3%	-15.6%	-19.8%	-13.1%
Wet food 8	-8.7%	-19.9%	5.5%	-0.7%
Wet food 9	16.4%	-1.2%	8.2%	21.5%
Wet food 10	-7.7%	4.5%	-36.5%	-21.2%
Average wet food	**4.2%**	**-4.8%**	**-13.3%**	**8.3%**
Dry food 1		-11.8%	0.0%	-4.8%
Dry food 2	4.9%	5.3%	3.7%	-4.7%
Dry food 3		7.3%	-8.0%	2.7%
Dry food 4		6.9%	2.3%	-11.2%
Dry food 5	1.8%	7.7%	6.7%	-10.5%
Dry food 6	3.6%	-0.4%	-33.6%	-16.1%
Dry food 7		2.7%	-22.0%	-12.4%
Dry food 8	0.8%	3.5%	-26.5%	-11.5%
Dry food 9	3.3%	8.0%	-10.0%	-6.7%
Dry food 10		4.3%	7.8%	19.0%
Average dry food	**2.9%**	**3.3%**	**-7.9%**	**-5.6%**

[1]Deviation=(measured-declared) x measured/100; Minus value indicates lower measured nutrient than declared in %, plus value indicates higher measured nutrient in %. The measured and declared nutrients were corrected by the respective DM content.

4.1.2 Differences in the nutrient composition among three foods

The results of the ANOVA are shown in Table 4 and Figure 1. As expected, the dry food had the highest DM content, with an estimated mean of 90.6±1.39 %. There was no significant difference in the DM content between the wet (22.7±1.39 %) and homemade (26.4±1.39 %)

food. On DM basis, the mean ash content of wet food (10.4±0.46 %) was significantly higher than in dry (7.25±0.49 %) and homemade (6.30±0.49 %) food. Wet food also had the highest estimated mean CP content (44.1±0.49 %). There was no significant difference in the CP content between dry (26.2±1.44 %) and homemade (29.1±1.44 %) food. The EE content of wet food (23.9±2.30 %) was significantly higher than in dry food (12.3±2.30 %) but was not significantly higher than the EE content of homemade food (18.8±2.30 %). Between the EE content of homemade and of dry food was no significant difference. The ADF content of homemade food (9.49±1.23 %) was significantly lower that the ADF content of wet (17.3±1.23 %) and dry (14.6±1.23 %) food. The NFC content of wet food (5.88±2.76 %) was significantly lower than in homemade (36.3±2.76 %) and dry (39.3±2.76 %) food.

The nutrient composition of each sample can be found in the appendix.

Tab. 5: Results of nutrient composition of different food types

	Tested food type			SEM	P-value
Variable [% DM unless stated]	Wet food	Homemade	Dry food		
DM [%]	22.7a	26.4a	90.6b	1.39	<0.001
Ash	10.4b	6.30a	7.25a	0.46	<0.001
Crude protein	44.1b	29.1a	26.2a	1.44	<0.001
Ether extracts	23.9a	18.8ab	12.3b	2.30	0.0052
ADF	17.3a	9.49b	14.6a	1.23	<0.001
NFC*	5.88b	36.3a	39.6a	2.76	<0.001

Within a row, the means with different letters differ according to Tukey test ($p<0.05$)

*NFC=100-(NDF+CP+EE+ash)

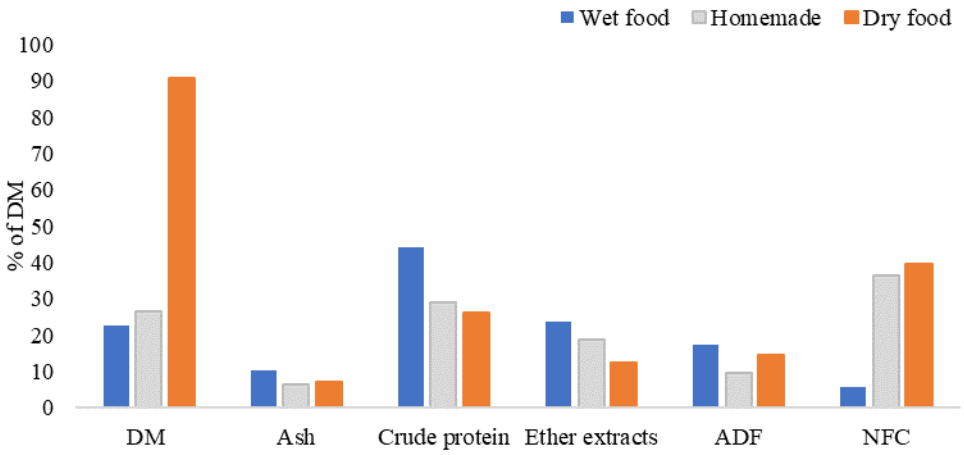

Fig. 1: Average nutrient composition of each dog food type

4.2 Buffering capacity of different food types

The results of the ANOVA are shown in Table 5. The undiluted pH of homemade dog food was significantly lower than the undiluted pH of wet food. There was a significant difference of the initial pH of all three food types. Wet food had, with an estimated mean of 6.77±0.08 the highest initial pH followed by homemade food, with 6.31±0.08 and dry food had with 5.62±0.08 the lowest pH. Dry food had, with 8.22±0.34, compared to wet and homemade food a significant higher BC. But if we look at the BC per gram dry matter (BC/g DM) the wet food had, with 2.72±0.14, a significantly higher BC/g DM compared to the other food types. Matching the result of the BC/g DM, wet food had also a significant higher used HCl per gram dry matter (HCl/g DM) than the other food types.

The results of the BC of each sample and the titration curves can be found in the appendix.

Tab. 6.: Buffering capacity of different food types

	Tested food type			SEM	P-value
Variable	Wet food	Homemade	Dry food		
Undiluted pH[1]	6.66a	5.98b		0.10	<0.001
Initial pH	6.77a	6.31b	5.62c	0.08	<0.001

Buffering Capacity (BC)[2]	3.02a	2.40a	8.22b	0.34	<0.001
Used HCl/g DM [ml]	13.2b	8.15a	6.69a	0.67	<0.001
BC/g DM	2.72b	1.86a	1.83a	0.14	<0.001

Within a row, the means with different letters differ according to Tukey test ($p<0.05$)

[1]Undiluted pH is the pH of the food without any addition of water

[2]BC=total acid added/(initial pH-final pH)

4.3 Associations between nutrients and variables of buffer capacity

Linear regression graphs were generated to evaluate effect of nutrients on used HCl per gram DM to reach pH < 2 as well as the measured BC of the food per gram DM. Figure 2 to 4 show linear and positive associations between nutrient composition of the dog food and the used HCl per gram DM. Accordingly, the data of Figure 1 revealed that 83 % of the variance of used HCl/g DM could be predicted with the percentage of crude protein in DM of dog food. According to this regression analysis, for each 1 % CP in the diet, each g food of DM ingested would require 0.34 ml HCl to reach a pH < 2. Also, the ash (R^2=0.57) was a good predictor for the usage of HCl/g DM, 1.16 ml HCl, for each g DM of food, were needed for each 1 % of Ash to reach pH < 2. Whereas the NCF (R^2=0.60) content had a negative effect on the amount of HCl needed. EE und ADF had no relevant effect on the used HCl per gram DM.

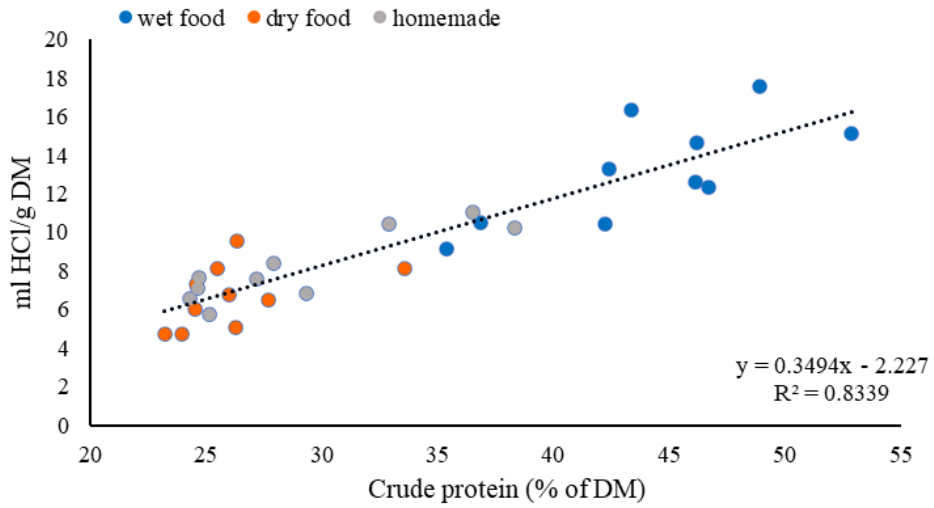

Fig. 2.: Effect of crude protein content in the diet on the amounts of HCl per gram DM wet, dry, and homemade food required to lower the pH < 2

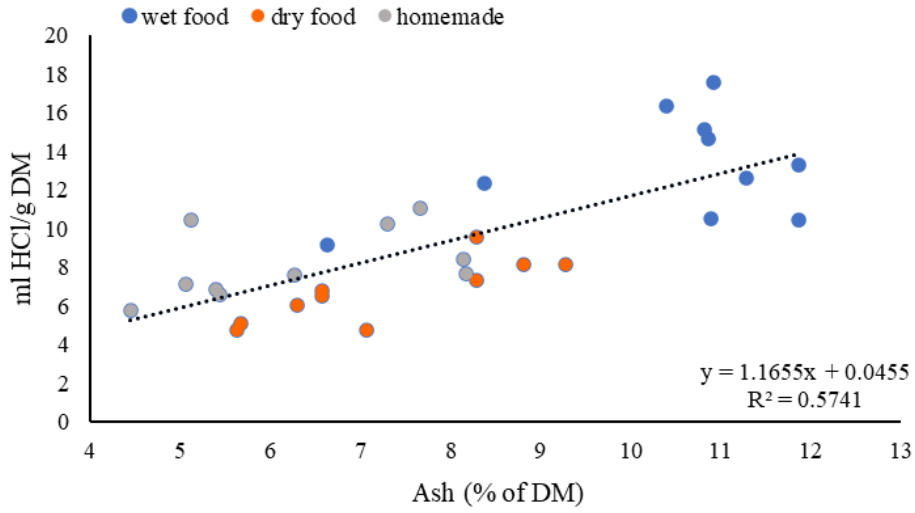

Fig. 3.: Effect of ash content in the diet on the amounts of HCl per gram DM wet, dry, and homemade food required to lower the pH < 2

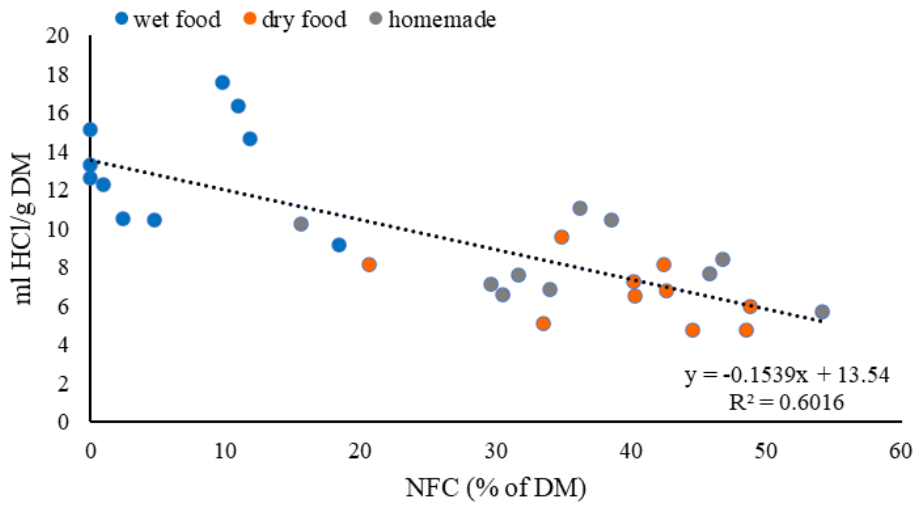

Fig. 4.: Effect of NFC content in the diet on amounts of HCl per gram DM wet, dry, and homemade food required to lower the pH < 2

Figure 5 shows a polynomial regression of degree 2 between used HCl per gram DM and initial pH. The coefficient of determination is 0.63. The initial pH correlated only positively with the amount of HCl for wet and homemade food, but less with dry food. The initial pH of dry does not seem to affect the amount of HCl pro g DM required to lower the pH < 2.

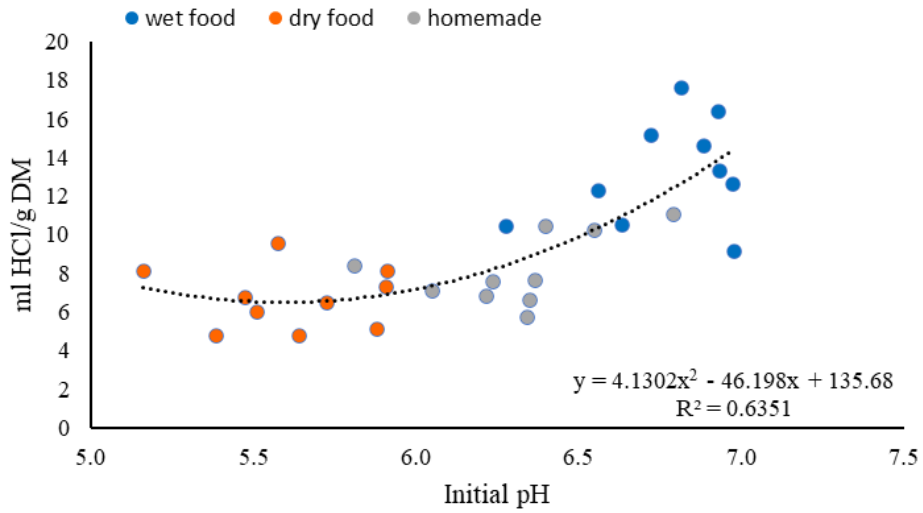

Fig. 5.: Effect of initial pH in the diet on amounts of HCl per gram DM wet, dry, and homemade food required to lower the pH < 2

Figure 6 to 8 show the linear regression between nutrient composition and of the dog food and BC per gram DM. Figure 5 shows that 72 % of the variance of BC/g DM could be predicted with the percentage of crude protein in DM of dog food. Increasing the protein content by 1 % led to an increase in BC/g DM of 0.06 Also, the ash (R^2=0.60) led to an increase in BC/g DM of 0.2 per % increase of ash content. Whereas the NCF (R^2=0.50) content in DM of dog food had a negative effect on the BC/g DM.

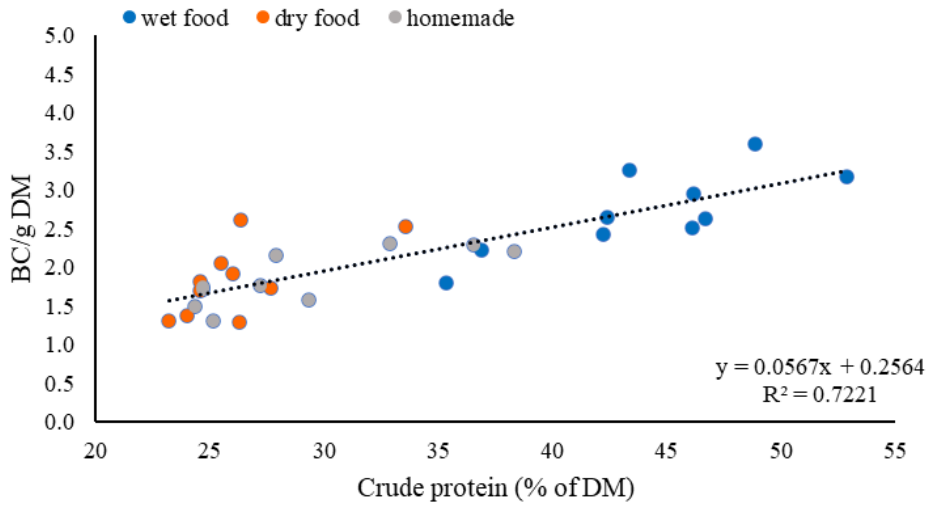

Fig. 6.: Effect of crude protein content on the BC per gram DM wet, dry, and homemade food

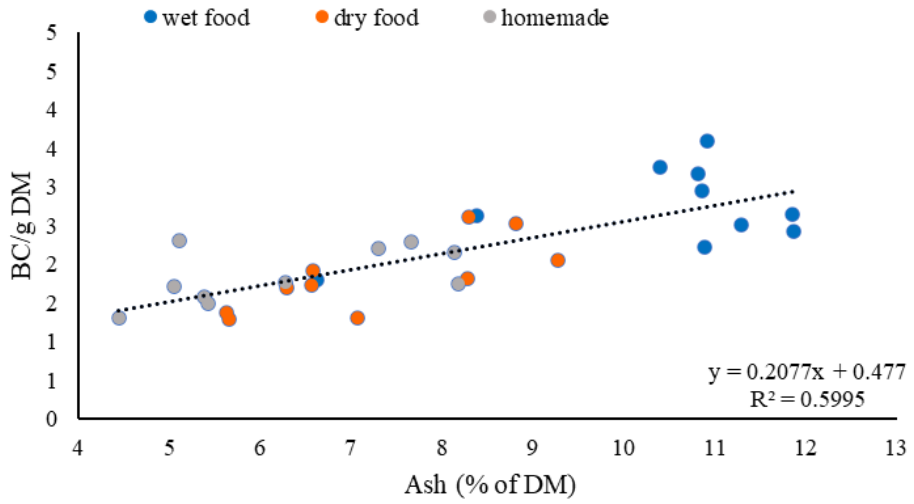

Fig. 7.: Effect of ash on the BC per gram DM wet, dry, and homemade food

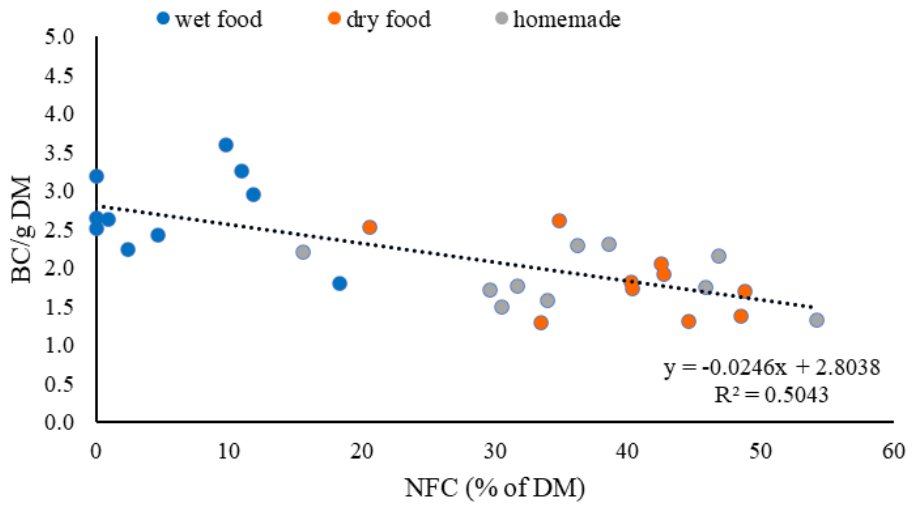

Fig. 8.: Effect of NFC on BC per gram DM wet, dry, and homemade food

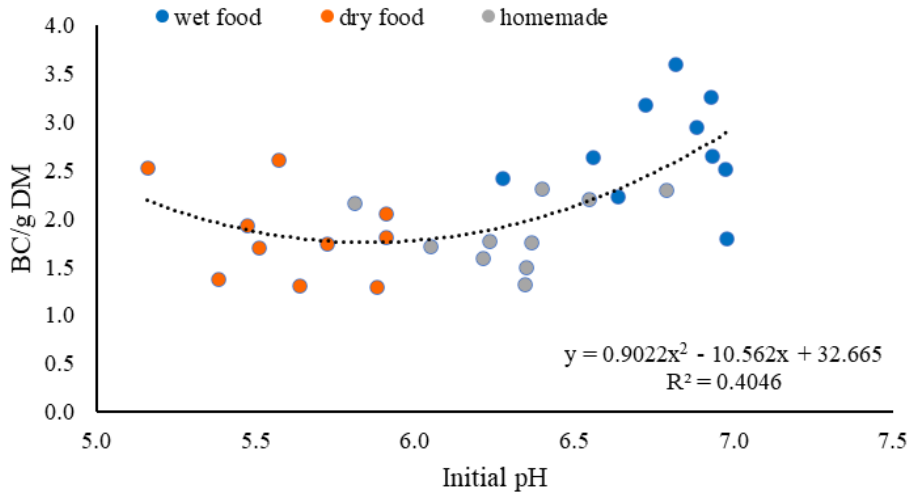

Fig. 9.: Effect of initial pH on BC per gram DM wet, dry, and homemade food

Figure 9 shows a polynomial regression of degree 2 between used HCl per gram DM and initial pH. The coefficient of determination (R^2) is 0.40. The initial pH correlated positively with the BC/g DM of wet and homemade food, but less with dry food. The initial pH of dry food does not seem to affect the BC/g DM.

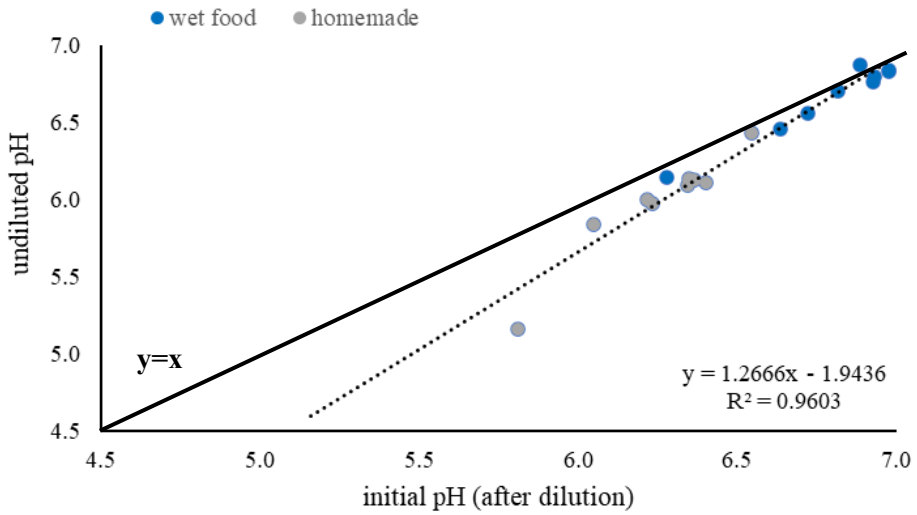

Fig. 10.: Relationship between undiluted pH of wet and homemade food and its initial pH (after dilution with distilled water)

Figure 10 shows the linear regression between undiluted pH and initial pH. The graphic shows that the effect of the addition of water to the dog food, the lower the pH of the food is the higher is the effect of the addition on water.

4.4 Multiple Regression

A multiple regression was performed to evaluate the joint influence of and discriminate among different potential factors on HCl use per gram DM and BC per gram DM of the food to reach a pH < 2. The results of the multiple regression are shown in Table 7 and 8.

Tab. 7: Multiple regression for the dependent variable HCl/g DM

Variable	Parameter Estimate	Standard error	P-value	VIF	Adj. R^2	Root MSE	Dependent mean
Intercept	-8.90	3.49	0.0169	0	0.85	1.34	9.34
Ash	0.33	0.17	0.0591	2.29			
Crude protein	0.23	0.05	0.0002	3.79			
Initial pH	1.25	0.68	0.0769	2.14			

The most significant positive influence on the HCl use per gram DM has the percentage of crude protein (p=0.0002) in the dog food, followed by the amount of crude ash (p=0.0591) and the initial pH of the sample (p=0.0769).

Tab. 8: Multiple regression for the dependent variable BC/g DM

Variable	Parameter Estimate	Standard error	P-value	VIF	Adj. R^2	Root MSE	Dependent mean
Intercept	4.33	1.76	0.0275	0	0.85	0.25	2.27
Crude protein	0.06	0.01	<0.001	2.32			
Initial pH	-0.50	0.27	0.0850	2.38			
DM content	-0.04	0.01	0.0155	1.61			

The most significant influence on the BC per gram DM has the percentage of crude protein (p<0.001) in the dog food, which affected positively the BC corrected per gram of food DM. In contrast, the DM content of the food (p=0.0155) and its initial pH (p=0.0850) both negatively affected the food BC corrected per gram DM.

5. Discussion

The aim of this study was to analyze the buffering capacity and the HCl amount needed to acidify the food, both as an indication of acidity of commercial dog food and homemade dog food in relation to their nutrient composition. Therefore, the buffering capacity of 30 complete dog foods, each ten different types of commercial dry and wet dog food and homemade dog food, was measured and parameters that influenced the buffering capacity were evaluated.

5.1 Differences in initial pH, BC and HCl use among the food types

In our study, we could observe differences in initial pH, BC/g DM and used HCl/g DM among the different food types.

The lowest initial pH had dry food with mean pH of 5.62 in a range from 5.16 to 5.91. Followed by homemade food with a mean pH of 6.31 in a range from 5.81 to 6.79. The highest pH had wet food with a mean pH of 6.77 in a range from 6.28 to 6.98. A possible explanation for the low initial pH in dry food could be found in the significantly lower CP content in dry compared to wet food. However, the CP content does not explain the significant difference in initial pH between dry and homemade food, because there was no significant difference in CP content. The difference between these two different food types could be explained through the different DM content and preservatives, like citric acid and mixed tocopherols, that are added to dry food. The difference between the initial pH of wet and homemade food could be explained through the different CP content (Giger-Reverdin et al. 2002). In this context, it is also interesting to mention that a low pH of dog food could possibly increase the risk of caries, because it lowers the pH of the saliva (Smeets-Peeters et al. 1998, Hale 2009).

There are also differences in the BC per gram DM among the food types. The highest BC per gram dry matter had wet food with a mean of 2.72 in a range from 1.79 to 3.59, followed by homemade food with a mean of 1.86 in a range of 1.50 to 2.31. The lowest BC per gram DM had dry food with a mean of 1.83, in a range from 1.29 to 2.60. It is no surprise that wet food had the highest BC per gram DM, because it also had the highest CP content (Montañez-Valdez

et al. 2013, Mennah-Govela et al. 2020). There was no significant difference in the BC per gram DM between dry and homemade food, this was due to the similar CP content of these two food types.

The highest use of HCl per gram DM had wet food, with a mean of 13.2 ml in a range from 9.14 to 17.57 ml, followed from homemade food with a mean of 8.15 ml, in a range from 5.73 to 11.03. The lowest use of HCl per gram DM had dry food with a mean of 6.69 ml in a range from 4.73 to 9.54 ml. According to our results from the multiple regression ash had a significant influence ($p=0.59$) on the HCl use and wet food had a significantly higher ash content than the other food types. This finding in combination with the high CP content of wet food, could explain the high HCl use.

For all food types, there was a wide range in the values for their own food type. This could be explained through their different nutrient composition, but also induvial ingredients could have an influence on the initial pH, the BC per gram DM and the HCl use per gram DM. However, measuring the BC commonly used ingredients in dog food would be necessary to prove the theory.

In view of the results the question arises whether the different food types have a different influence on gastric pH in dogs. There are no studies that measured the gastric pH of dogs during digestion and provided information of the BC of the used food. However, it is possible to calculate with our data the amount of gastric acid needed and time to raise the gastric pH under two. Dogs have postprandial gastric acid secretion from 1.5 ml per kg body mass per minute (Zentek 2022). For instance, if we take a 10 kg dog that is feed a meal with 70 g of DM dog food and take the mean values of each food type, for wet food 924 ml gastric acid and 62 minutes are needed to reach a gastric pH under 2. In comparison for homemade food 570 ml gastric acid and 47 minutes, whereas for dry food 468 ml gastric acid and 32 minutes are needed to reach a gastric pH under 2. It is important that the calculated values cannot be applied one-to-one to the in vivo conditions in the stomach since the gastric pH is influenced by many other factors too.

This information could be important for dogs with gastric hypoacidity or gastrits to choose the right food, where a small amount of gastric acid is needed to reach the right pH levels in the stomach.

5.2 Differences in nutrient composition among the food types

Significant differences between the nutrient composition could be found among the food types. Wet food had the highest CP content with a mean of 44.1 % and was therefore significantly higher than from homemade (mean 29.1 % DM) and dry food (mean 26.2 % DM). In another study that evaluated the nutrient composition of commercial dry and wet dog food an average CP of 28.7 % DM in dry and 39.3 % DM in wet food was found (Sgorlon et al. 2022). The difference in CP content in dry and wet dog food could be explained through different demand on the manufacturing process of the food. A high level of starch is needed in dry food to maintain the durability of the kibble (Manbeck et al. 2017), the production process of wet food does not require starch, so it mostly contains meat and offal (Sgorlon et al. 2022). The rather low CP content of homemade food compared with wet food was due to the decision to feed rations with a low protein content, enough to cover the dog requirements in CP, but to cover the energy requirements mainly via carbohydrates and fats.

The highest EE content had wet food with mean of 23.9 % DM, followed by homemade food (mean 18.8 % DM) and dry food (mean 12.3 % DM). Surprisingly, the measured EE content in wet food was on average 13.3 % lower and in dry food 7.9 % lower than declared by the manufactures. Differences in the EE content could be caused by a different fat content of different parts of the carcass that were used for the dog food.

Also, the different NFC content between the food types can be explain with the manufacturing process. The NFC provides information about the amount of cell ingredients, mostly starch and sugar, and pectin in a diet (Bellof et Granz 2019)This explains the high NFC of dry food, with a mean of 39.9 % DM and homemade food, with a mean of 36.3 % DM.

In our study wet food had mean ash content of 10.4 % DM, dry food 7.25 % DM and homemade food 6.3 % DM. Furthermore, it is interesting to mention than in our study the measured ash

content of wet food was on average 8.3 % higher than declared by the manufacturers. These results are quite similar to another study that measured an average ash content in dry food of 7.34 % DM and in wet food of 10.1 % DM (Davies et al. 2017). Specifically, the high ash content in wet food could be a the risk factor for a gastric dilatation-volvulus syndrome, because it could lead to an insufficient pH drop in the stomach, which promotes the growth of gas producing bacteria (Zentek 2022). A possible explanation for the high ash content of wet food gives Sgorlon et al. (2022) as they mentioned that higher concentration of Na and K was measured in wet food. NaCl is often added in wet food to increase the acceptance and palatability, and the addition of Na alginate, K alginate or K carrageenin as gelling agent and thickeners (Sgorlon et al. 2022). Yet, as observed in this study, ash increases BC of wet food significantly; therefore, our data suggest that the use of gelling agents in the wet food needs additional evaluation.

5.3 Influence of protein on BC and HCl use

In our study, the content of CP was the most important factor that influenced the buffering capacity of dog food. In the multiple regression the CP had very strong and positive significant influence on the BC/g DM and used HCl/g DM. Unfortunately, we do not have information about the exact protein composition of the tested food. Other studies have already shown that different proteins sources, e. g. plant-based or animal origin) and different parts of the carcass, e. g. chicken skin or meat, have different BC values (Tan et al. 2014, Ebert et al. 2021).

Also, other studies described protein as one of the main factors that affected the BC of food. For example, Mennah-Govela et al. measured the BC of thirty commercially available commonly used food products, that could be eaten as purchased, for example milk, canned chicken, tofu, in her study the protein content correlated with the total BC (R^2=0.67) and the total acid added (R^2=0.82) (Mennah-Govela et al. 2020). These results are similar to our results were the crude protein content correlated with BC/g DM (R^2=0.72) and HCl/g DM (R^2=0.83). Another study which measured the BC of commonly used feedstuff in ruminant diets concluded that the BC was high when the crude protein was high (>15 %) and decreased as the protein

content decreased (Montañez-Valdez et al. 2013). Wet food had on our study the highest content of crude protein in DM with 44.1% compared to dry food (26.2 %) and homemade food (29.1 %) and was therefore significant higher. The BC/g DM and used HCl/g DM was also significantly higher in wet food than in other food types. Further studies that analysed the BC of commonly used ingredients of pig and poultry feed came also to the conclusion that the protein content was an important factor (Lawlor et al. 2005, Levic et al. 2005).

5.4 Influence of ash on BC and HCl use

After performing a multiple regression, the ash content of the food had only a weak significant effect on the used HCl/g DM with a $p=0.0591$. A significant effect of crude ash content on BC/g DM was not proved after multiple regression. In the linear regression between BC/g DM and crude ash R^2 was 0.60 and between used HCl/g DM R2 was 0.57. Jasaitis et al. analysed in his study the BC of fifty-two feeds, representing common ingredients used in ruminant diets and found a significant correlation between BC ash content ($p<0.001$) (Jasaitis et al. 1987). The difference in the results of both studies could be due to a different cation-anion ratio in the ash content from dog food and ruminant feed. Anions also interact differently with different minerals, different composition of minerals could also be a reason for the different results (Salaün et al. 2005).

5.5 Influence of the initial pH on BC and HCl use

In our study wet food had the highest initial pH with a mean of 6.77 followed by homemade food with a mean on 6.31 and dry food with a mean of 5.62. The initial pH had only a weak negative significant influence on the BC/g DM with a $p=0.0850$ and on used HCl/g DM a weak positive influence, with a $p=0.0769$. In another study the initial pH had a negative significant influence on the BC ($p<0.05$) but no significant influence on the total acid added ($p>0.05$) (Mennah-Govela et al. 2020b).

The protein content of feedstuff had an influence on the initial pH, a study identified that feedstuff with a high protein content have an initial pH around neutrality (Giger-Reverdin et al. 2002). Wet food had the highest protein content in DM and the highest initial pH. Homemade food had no significant different protein content in DM compared to dry food but had a significant higher initial pH. Giger-Reverdin emphasized in her study that feedstuffs which retained water hat had lower initial pH values than feedstuffs of the same protein content, but with a lower water holding capacity. Also, the preservatives of dry food could be a reason for the lower initial pH of dry food. Preservatives are added to the food to inhibit the growth of pathogens (Qu 2017). Another study that measured the pH of dry food came to similar results, there the pH of dry food ranged from 4.71 to 6.03 (Qu 2017).

5.6 Influence of DM content on BC

Dry food had with a mean DM content of 90.6 % a higher DM content than wet food (22.7 %) and homemade food (26.4 %). Also, the BC of dry food (8.22) was higher than the BC of wet food (3.02) and homemade food (2.40). These values could be explained though the measurement and calculation method of the BC. For the BC analysis, samples of 5 g from each food were used and the DM content of each food was not considered. However, if the BC was corrected for the DM, the DM content was considered, wet food had with a mean of 2.72 a significantly higher BC/g DM than dry food (1.83) and homemade food (1.86). This aspect shows why it is so important to compare the different feeds on a dry matter basis, otherwise the results would be misinterpreted. However, the multiple regression proved a negative significant influence ($p<0.05$) of DM content of the tested food on the BC/g DM. This means that the more water the food contains per gram DM, the higher is the BC/g DM.

5.7 Influence of particle size on BC

According to the research of Mennah-Govela the particle size had a significant influence on food with a protein content higher than 19 %, a CP content which is relevant to dog food.

Accordingly, a smaller particle size resulted in a higher buffering capacity (Mennah-Govela et al. 2020b). In our study the samples were mixed to obtain a comparable texture of the different dog feed types. However, the particle size could be important for the in vivo BC of dog food. Dry food had depending on the brand a different croquette size. Also, in homemade diets the particle size could play a role for the BC. For example, it could have an influence on the BC if minced meat is fed or meat sliced in pieces is fed. The possible influence of particle size on the BC of different dog feed types needs further research, tough.

5.8 Conclusion

In our study the CP content was the most important factor that influenced the BC and HCl use, whereas the initial pH had only a weak significant influence. Ash had only a weak significant influence on the used HCl. Wet food had the highest BC/g DM, between dry and homemade food was no significant difference of the BC/g DM. Our hypothesis that protein has an influence on BC was confirmed. A possible influence of particle size of dog food on the BC needs further research. Knowing the BC of dog food might be important to predict outcomes like gastric digestion, events which are of major importance for dogs that have problems with gastric hypoacidity or gastritis. For dogs with these disorders, a declaration of the BC on the label could be helpful in selecting the proper food, especially for manufacturers who produce special diets for dogs with gastrointestinal disorders. Dog owners and veterinarians can estimate the BC from protein and ash content of the food. A high protein and ash content indicates a high BC of the food. But more research about the influence of dog food BC on gastric pH in vivo is needed. In conclusion, the BC of food is a potentially interesting parameter for the future as it can provide important information about the effect of food on digestion, and it can help to select the right diet for the needs of dogs.

6. Summary

6.1 Summary English

Buffering capacity (BC) is defined as the resistance of a solution to change pH with the addition of an acid or a base. Therefore, the buffering capacity of food could have an influence on the gastric pH of dogs. The gastric pH plays an important role for the activity of enzymes and acts as a barrier against foodborne pathogens. Dog food with a high buffering capacity could have a negative effect on the digestion, because the pH in the stomach remains too high. Based on the results of previous studies it is believed that the protein content of dog food influences the BC.

To check the hypothesis the buffering capacity of 30 complete dog food rations, each ten different types of commercial dry and wet dog food and ten homemade dog food rations, were quantified. The measurement of the BC was done with acid titration method. Of each food samples were prepared, each sample consisted of 5 g dog food that was mixed with 15 g of distilled water. Under continuous stirring 0.16 M HCl was added to the sample until the pH was under 2. After each addition of 1 ml HCl to the sample the pH was measured with a portable pH meter. Based on the data of the acid titration the BC was calculated. Using multiple regression, the aim was to establish associations between the nutrient composition of the dog food and the BC of the dog food. Protein content had a highly significant ($p<0.001$), whereas the initial pH had only a weak influence ($p<0.1$) on the BC und used HCl per gram dry matter (DM). Additionally, the crude ash content had a weak influence on the used HCl per gram DM and the DM content had a significant ($p<0.05$) influence on the BC per gram DM.

Our hypothesis that protein has an influence on BC was confirmed. Knowing the BC of dog food could be important for dog that have problems with gastric hypoacidity or gastritis. But more research about the influence of dog food BC on gastric pH in vivo is needed.

6.2 Summary German

Pufferkapazität ist definiert als die Fähigkeit einer Lösung die Zugabe einer Säure oder Base abzufangen, ohne den pH-Wert dabei zu verändern. Die Pufferkapazität von Hundefutter könnte einen Einfluss auf den pH-Wert der Magensäure haben. Diese spielt eine wichtige Rolle für die Enzymaktivität und agiert als wichtige Barriere gegen lebensmittelbedingte Krankheitserreger. Hundefutter mit einer hohen Pufferkapazität könnte sich negativ auf die Verdauung auswirken, da der pH-Wert des Magens nicht ausreichend gesenkt wird und die Enzyme nicht ihre optimale Wirkung entfalten können. Basierend auf Ergebnissen von bisherigen Studien wird vermutet, dass der Proteingehalt von Hundefutter einen Effekt auf die Pufferkapazität hat.

Um diese Hypothese zu überprüfen, wurde die die Pufferkapazität von 30 verschiedenen Hundefuttersorten, jeweils zehn verschiedene Trocken- und Nassfuttersorten, sowie zehn selbst gekochten Rationen, gemessen. Die Pufferkapazität wurde mittels Säuretitration gemessen. Dafür wurden Proben bestehend aus 5 g Futter und 15 g destillierten Wasser hergestellt. Unter ständigem Rühren wurde der pH-Wert der Probe, jedes Mal nach der Zugabe von 1 ml 0,16 M Salzsäure gemessen. Dieser Vorgang wurde so lange wiederholt bis der pH-Wert der Probe unter 2 lag. Mit der den Daten der Titration wurde die Pufferkapazität gemessen. Mittels Multipler Regression wurde versucht ein Zusammenhang zwischen der Zusammensetzung der Rohnährstoffe von Hundefutter und der Pufferkapazität zu finden. Der Proteingehalt hatte einen hoch signifikanten ($p<0.001$) Einfluss hab die Pufferkapazität und der verwendete Salzsäuremenge pro Gramm Trockensubstanz, während der initiale pH der Probe nur einen schwach signifikanten ($p<0.1$) Einfluss auf diese hatte. Zusätzlich hatte der Rohaschegehalt einen schwach signifikanten Einfluss auf die verwendete Salzsäuremenge pro Gram Trockensubstanz (TS). Der Trockensubstanzgehalt des Hundefutters hatte einen signifikanten ($p<0.05$) Einfluss auf die Pufferkapazität pro Gram TS.

Unsere Hypothese, dass der Proteingehalt von Hundefutter einen Einfluss auf die Pufferkapazität hat, wurde bestätig. Die Pufferkapazität des Futters zu wissen, könnte für Hunde, die an Hypoazidität oder Gastritis leiden wichtig sein. Allerdings braucht es mehr Forschung über die Wirkung von Pufferkapazität von Hundefutter in vivo

List of abbreviation

ADF	Acid detergent fibre
ANOVA	Analysis of variance
BC	Buffering Capacity
BC/g DM	Buffering Capacity per gram dry matter
CP	Crude protein
DCAB	Dietary Cation Anion Balance
DM	Dry Matter
EE	Ether extracts
HCl	Hydrochloride acid
NDF	Neutral detergent fibre
NFC	Non-fiber carbohydrates
R^2	Coefficient of determination
rpm	Rotations per minute

References

Akimoto M, Nagahata N, Furuya A, Fukushima K, Higuchi S, Suwa T. 2000. Gastric pH profiles of beagle dogs and their use as an alternative to human testing. European Journal of Pharmaceutics and Biopharmaceutics, 49(2):99–102.

Al-Dabbas MM, Al-Ismail K, Taleb RA, Ibrahim S. 2010. Acid-base buffering properties of five legumes and selected food in vitro. American Journal of Agricultural and Biological Science, 5(2):154–160.

Banta CA, Clemens ET, Krinsky MM, Sheffy BE. 1979. Sites of organic acid production and patterns of digesta movement in the gastrointestinal tract of dogs. Journal of Nutrition, 109(9):1592–1600.

Bellof G, Granz S. Hrsg. 2019. Tierproduktion : Nutztiere züchten, halten und ernähren. 15. Aufl. Stuttgart: Georg Thieme Verlag.

Breves G, Diener M, Gäbel G. Hrsg. 2022. Physiologie der Haustiere. 6. Aufl. Stuttgart: Georg Thieme Verlag.

Brooks AM, Grossman MI. 1970a. Postprandial pH and neutralizing capacity of the proximal duodenum in dogs. Gastroenterology, 59(1):85–89.

Brooks AM, Grossman MI. 1970b. Effect of secretin on fasting and postprandial pH of the duodenal bulb in dogs. Gastroenterology, 59(1):90–94.

Brooks FP. 1985. Effect of diet on gastric secretion. The American Journal of Clinical Nutrition, 42(5):1006–1019.

Carrière F, Raphel V, Moreau H, Bernadac A, Devaux MA, Grimaud R, Barrowman JA, Bénicourt C, Junien JL, Laugier R, et al. 1992. Dog gastric lipase: Stimulation of its secretion in vivo and cytolocalization in mucous pit cells. Gastroenterology, 102(5):1535–1545.

Carrière F, Moreau H, Raphel V, Laugier R, Benicourt C, Junien J -L, Verger R. 1991. Purification and biochemical characterization of dog gastric lipase. European Journal of

Biochemistry, 202(1):75–83.

Constable PD. 1999. Clinical assessment of acid-base status. Strong ion difference theory. The Veterinary clinics of North America. Food animal practice, 15(3):447–471.

Ebert S, Baune MC, Broucke K, Royen G Van, Terjung N, Gibis M, Weiss J. 2021. Buffering capacity of wet texturized plant proteins in comparison to pork meat. Food Research International, 150(PB):110803.

Garcia-Mazcorro JF, Suchodolski JS, Jones KR, Clark-Price SC, Dowd SE, Minamoto Y, Markel M, Steiner JM, Dossin O. 2012. Effect of the proton pump inhibitor omeprazole on the gastrointestinal bacterial microbiota of healthy dogs. FEMS Microbiology Ecology, 80(3):624–636.

Giger-Reverdin S, Duvaux-Ponter C, Sauvant D, Martin O, Nunes do Prado I, Müller R. 2002. Intrinsic buffering capacity of feedstuffs. Animal Feed Science and Technology, 96(1–2):83–102.

Hale FA. 2009. Dental caries in the dog. The Canadian veterinary journal = La revue veterinaire canadienne, 50(12):1301–1304.

Hinder RA, Kelly KA. 1977. Canine gastric emptying of solids and liquids. The American journal of physiology, 233(4):E335-40.

Hornof WJ, Koblik PD, Strombeck DR, Morgan JP, Hansen G. 1989. Scintigraphic Evaluation of Solid-Phase Gastric Emptying in the Dog. Veterinary Radiology, 30(6):242–248.

Horowitz M, Dent J, Fraser R, Sun W, Hebbard G. 1994. Role and integration of mechanisms controlling gastric emptying. Digestive diseases and sciences, 39(12 Suppl):7S-13S.

Iacopetti I, Perazzi A, Badon T, Bedin S, Contiero B, Ricci R. 2017. Salivary pH, calcium, phosphorus and selected enzymes in healthy dogs: a pilot study. BMC veterinary research, 13(1):330.

Itoh Z, Honda R, Aizawa I. 1980. Diurnal pH changes in duodenum of conscious dogs. The American journal of physiology, 238(2).

Jasaitis DK, Wohlt JE, Evans JL. 1987. Influence of Feed Ion Content on Buffering Capacity of Ruminant Feedstuffs In Vitro. Journal of Dairy Science, 70(7):1391–1403.

Kararli TT. 1995. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. Biopharmaceutics & Drug Disposition, 16(5):351–380.

Kienzle E. 1988. Enzymaktivität in Pancreas, Darmwand und Chymus des Hundes in Abhängigkeit von Alter und Futterart. Journal of Animal Physiology and Animal Nutrition, 60(1–5):276–288.

Kues WA, Köckritz-Blickwede M von, Branitzki-Heinemann KC, Brogden G, Buhr N de, Fehr M, Geburek F, Hennig-Pauka I, Naim HY, Steinhagen D. 2021. Biochemie für die Tiermedizin.

Lawlor PG, Lynch PB, Caffrey PJ, Lawlor PG. 2005. Peer Reviewed Pig Diets. Canadian Journal of Animal Science, 58(8):447–452.

Levic J, Prodanovic O, Sredanovic S. 2005. Understanding the buffering capacity in feedstuffs. Biotechnology in Animal Husbandry, 21(5–6):309–313.

Luo Q, Zhan W, Boom RM, Janssen AEM. 2018. Interactions between acid and proteins under in vitro gastric condition-a theoretical and experimental quantification. Food and Function, 9(10):5283–5289.

Mahar KM, Portelli S, Coatney R, Chen EP. 2012. Gastric pH and Gastric Residence Time in Fasted and Fed Conscious Beagle Dogs using the Bravo® pH System. Journal of Pharmaceutical Sciences, 101(7):2439–2448.

Maskell IE, Johnson J V. 1993. Digestion and absorption. The Waltham book of companion animal nutrition. Pergamon Press, New York.

Mennah-Govela YA, Bornhorst GM. 2021. Food buffering capacity: quantification methods and its importance in digestion and health. Food and Function, 12(2):543–563.

Mennah-Govela YA, Singh RP, Bornhorst GM. 2019. Buffering capacity of protein-based model food systems in the context of gastric digestion. Food and Function, 10(9):6074–

6087.

Mennah-Govela YA, Cai H, Chu J, Kim K, Maborang MK, Sun W, Bornhorst GM. 2020. Buffering capacity of commercially available foods is influenced by composition and initial properties in the context of gastric digestion. Food and Function, 11(3):2255–2267.

Meyer JH, Way LW, Grossman MI. 1970. Pancreatic bicarbonate response to various acids in duodenum of the dog. The American journal of physiology, 219(4):964–970.

Montañez-Valdez O, Solano-Gama J, Tinajero JJ, Guerra-Medina C, Coss A, Orozco-Hernández J-R. 2013. Buffering capacity of common feedstuffs used in ruminant diets. Revista Colombiana de Ciencias Pecuarias, 26:37–41.

Pali-Schöll I, Untersmayr E, Klems M, Jensen-Jarolim E. 2018. The effect of digestion and digestibility on allergenicity of food. Nutrients, 10(9):1–16.

Patel PK, Patel SK, Dixit SK, Rathore RS. 2018. Gastritis and Peptic Ulcer Diseases in Dogs: A Review. International Journal of Current Microbiology and Applied Sciences, 7(03):2475–2501.

Rérat M, Philipp A, Hess H, Liesegang A. 2010. Das Dietary Cation-Anion Difference (DCAD)-Konzept bei der Milchkuh.

Rothuizen J, de Vries-Chalmers Hoynck van Papendrecht R, van den Brom WE. 1990. Post prandial and cholecystokinin-induced emptying of the gall bladder in dogs. The Veterinary record, 126(20):505—507.

Rückert C. 2015. Harnsteinerkrankungen bei Hund und Katze – Welche diätetischen Maßnahmen sind sinnvoll? Kleintier Konkret, 18(06):10–16.

Sagawa K, Li F, Liese R, Sutton SC. 2009. Fed and fasted gastric pH and gastric residence time in conscious beagle dogs. Journal of Pharmaceutical Sciences, 98(7):2494–2500.

Salaün F, Mietton B, Gaucheron F. 2005. Buffering capacity of dairy products. International Dairy Journal, 15(2):95–109.

Van Slyke DD. 1922. on the Measurement of Buffer Values and on the Relationship of Buffer

Value To the Dissociation Constant of the Buffer and the Concentration and Reaction of the Buffer Solution. Journal of Biological Chemistry, 52(2):525–570.

Smeets-Peeters M, Watson T, Minekus M, Havenaar R. 1998. A review of the physiology of the canine digestive tract related to the development of in vitro systems . Nutrition Research Reviews, 11(1):45–69.

Steiner JM, Berridge BR, Wojcieszyn J, Williams DA. 2002. Cellular immunolocalization of gastric and pancreatic lipase in various tissues obtained from dogs. American Journal of Veterinary Research, 63(5):722–727.

Steiner JM [MitwirkendeR], Allenspach K [MitwirkendeR]. 2011. Gastroenterologie bei Hund und Katze : Klinik - Diagnostik - Therapie. Hannover : Schlütersche.

Suchodolski JS. 2016. Diagnosis and interpretation of intestinal dysbiosis in dogs and cats. Veterinary Journal, 215:30–37.

Tan SM, Lee SM, Dykes GA. 2014. Fat contributes to the buffering capacity of chicken skin and meat but enhances the vulnerability of attached Salmonella cells to acetic acid treatment. Food Research International, 66:417–423.

Theodorakis MC. 1980. External scintigraphy in measuring rate of gastric emptying in beagles. The American journal of physiology, 239(1).

Traynor OJ, Dozois RR, DiMagno EP. 1984. Canine interdigestive and postprandial gallbladder motility and emptying. American Journal of Physiology - Gastrointestinal and Liver Physiology, 9(4).

Walsh JH. 1988. Peptides as Regulators of Gastric Acid Secretion. Annual Review of Physiology, 50(1):41–63.

Wyse C a, Mclellan J, Dickie a M, Preston T, Yam PS. 2003. Emptying in the Dog and Cat : 1898 – 2002. Journal of Veterinary Internal Medicine, 17(©2 mm):609–621.

Yamka RM, Mickelsen SL. 2006. The Prediction of Urine pH Using Dietary Cations and Anions in Dogs Fed Dry and Wet Foods. The International Journal of Applied Research in Veterinary Medicine, 4(4):355–361.

Zentek J. 2022. Ernährung des Hundes. 9. Aufl. Stuttgart: Georg Thieme Verlag.

List of figures

List of tables

Appendix

Acid titration curves

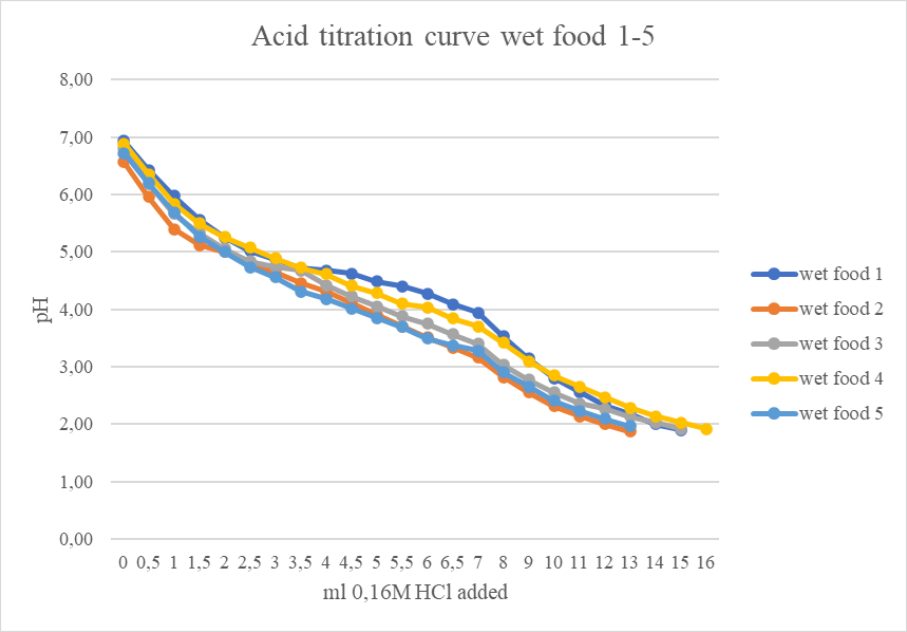

Fig. 11: Acid titration curve of wet food one to five

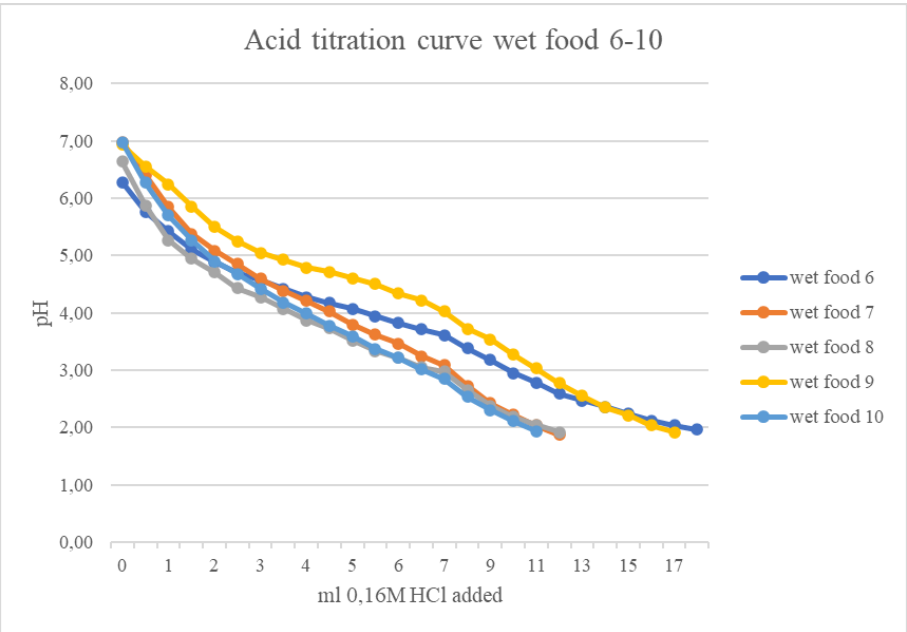

Fig. 12: Acid titration curves of wet food six to ten

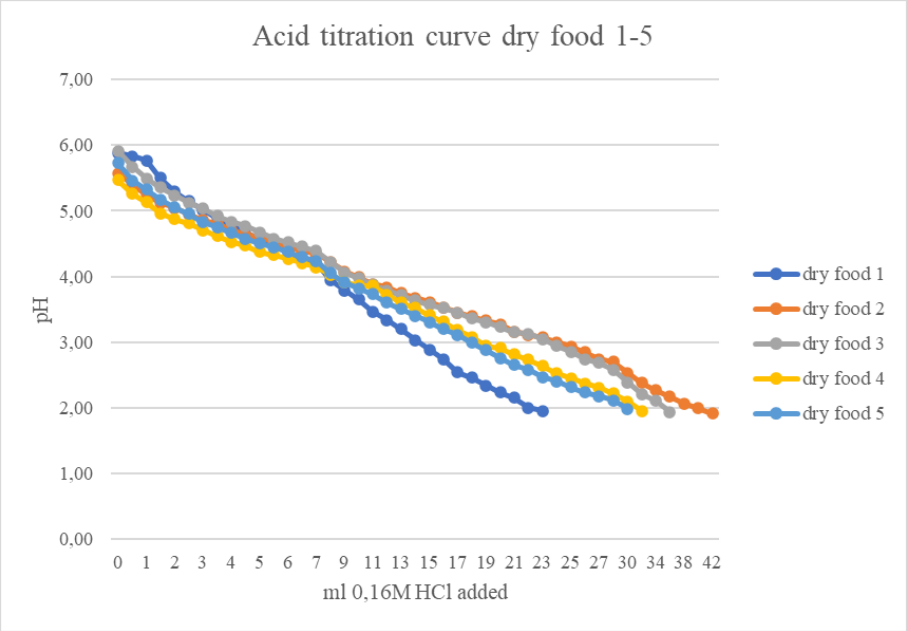

Fig. 13: Acid titration curves of dry food one to five

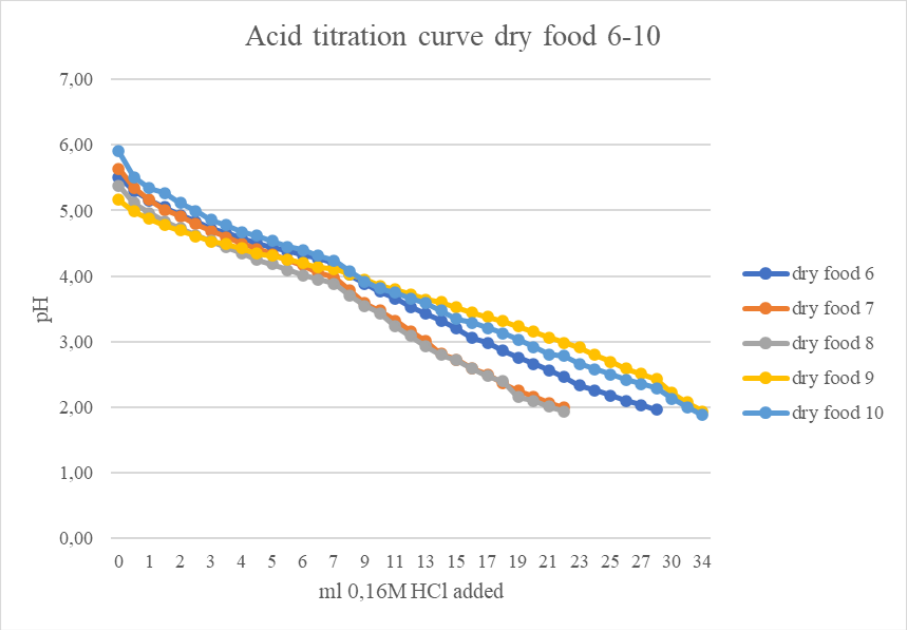

Fig. 14: Acid titration curves of dry food six to ten

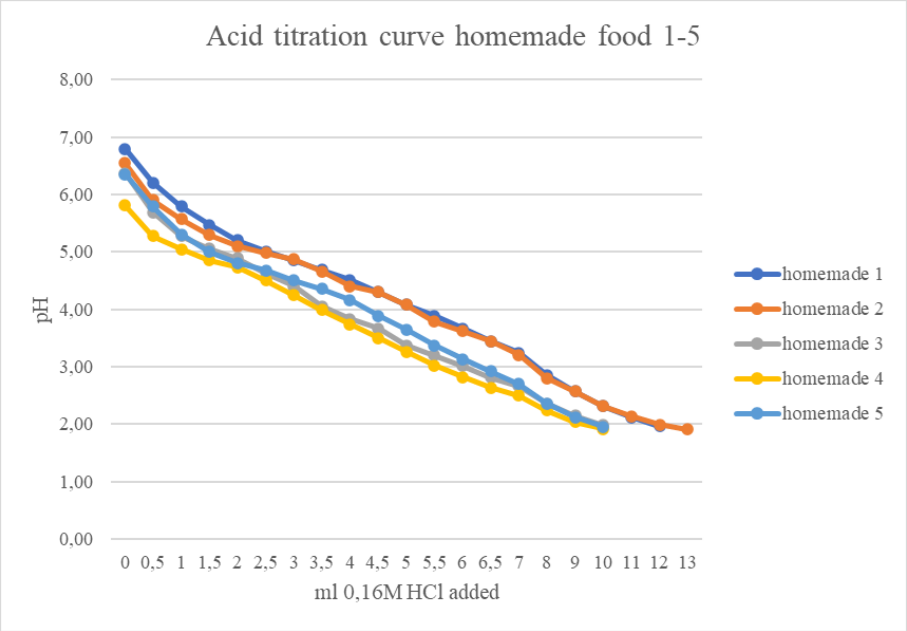

Fig. 15: Acid tirtation curves of homemade food one to five

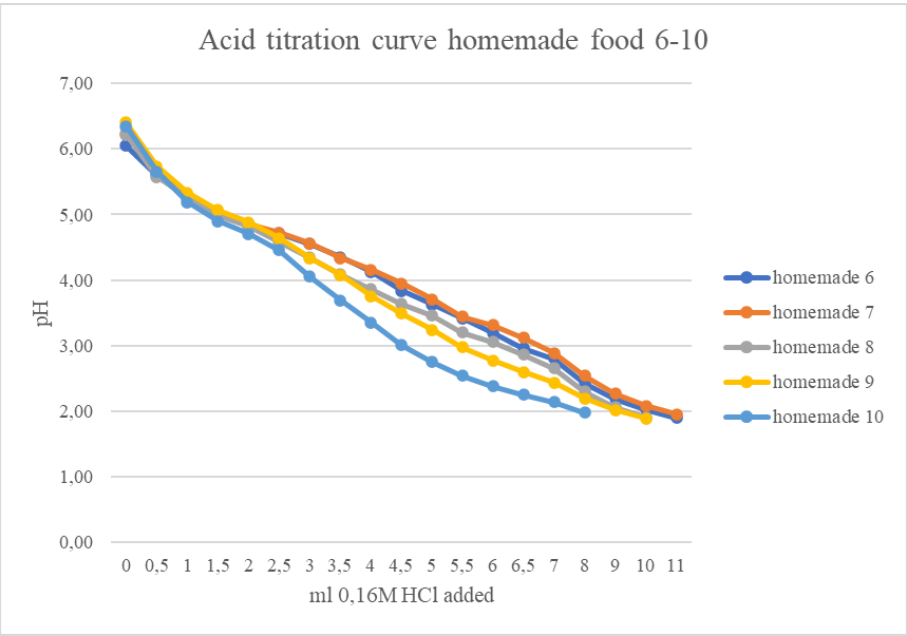

Fig. 16: Acid titration curves of homemade food six to ten

Nutrient composition of each sample

Tab. 9: Nutrient composition of each sample

ID	DM content	Ash	CP	EE	ADF	NFC
	% of DM					
Wet food 1	18.33	10.40	43.34	24.05	11.30	10.91
Wet food 2	21.13	8.38	46.69	30.29	13.71	0.92
Wet food 3	17.07	10.91	48.88	15.31	15.16	9.75
Wet food 4	21.88	10.86	46.19	18.65	12.49	11.82
Wet food 5	22.49	10.82	52.87	18.81	20.10	0.00
Wet food 6	34.53	11.87	42.24	26.10	15.12	4.67
Wet food 7	26.25	6.62	35.36	19.85	19.80	18.36
Wet food 8	22.82	10.88	36.86	37.00	12.89	2.37
Wet food 9	25.62	11.86	42.41	29.58	21.31	0.00
Wet food 10	17.44	11.29	46.11	20.03	31.09	0.00
Dry food 1	90.74	5.67	26.25	17.63	16.96	33.49
Dry food 2	83.90	8.29	26.35	14.84	15.71	34.81
Dry food 3	88.49	9.28	25.46	10.40	12.41	42.45
Dry food 4	94.55	6.58	26.01	11.91	12.87	42.64
Dry food 5	92.61	6.57	27.68	14.05	11.38	40.31
Dry food 6	93.28	6.30	24.55	7.48	12.90	48.77
Dry food 7	92.93	7.07	23.21	10.91	14.28	44.52
Dry food 8	92.74	5.63	23.99	8.32	13.54	48.51
Dry food 9	83.64	8.82	33.56	17.22	19.82	20.60
Dry food 10	93.36	8.29	24.57	10.39	16.55	40.20
Homemade 1	21.76	7.67	36.51	9.46	10.12	36.24
Homemade 2	25.46	7.30	38.33	26.14	12.70	15.54
Homemade 3	26.15	8.18	24.69	11.98	9.34	45.81
Homemade 4	23.85	8.14	27.89	9.46	7.74	46.77
Homemade 5	30.41	5.43	24.33	32.32	7.39	30.53
Homemade 6	31.01	5.06	24.66	33.04	7.66	29.58
Homemade 7	29.04	6.27	27.19	24.81	10.08	31.65
Homemade 8	29.31	5.39	29.34	22.57	8.74	33.96
Homemade 9	19.19	5.11	32.88	12.41	11.07	38.53
Homemade 10	27.93	4.45	25.16	6.17	10.08	54.14

Buffering capacity and used HCl of each sample

Tab. 10: Buffering capacity and used HCl of each sample

ID	pH undiluted	Initial pH	BC	Used HCl	DM content	Sample amount DM	HCl/g DM	BC/g DM
				ml	%	g	ml/g DM	g/DM
Wet food 1	6.76	6.93	2.98	15.00	18.33	0.92	16.36	3.26
Wet food 2	.	6.56	2.77	13.00	21.13	1.06	12.30	2.62
Wet food 3	6.70	6.82	3.06	15.00	17.07	0.85	17.57	3.59
Wet food 4	6.87	6.89	3.22	16.00	21.88	1.09	14.62	2.94
Wet food 5	6.56	6.72	3.57	17.00	22.49	1.12	15.12	3.18
Wet food 6	6.14	6.28	4.17	18.00	34.53	1.73	10.43	2.42
Wet food 7	6.83	6.98	2.35	12.00	26.25	1.31	9.14	1.79
Wet food 8	6.45	6.64	2.54	12.00	22.82	1.14	10.52	2.23
Wet food 9	6.79	6.93	3.39	17.00	25.62	1.28	13.27	2.64
Wet food 10	6.84	6.98	2.18	11.00	17.44	0.87	12.61	2.50
Dry food 1	.	5.88	5.86	23.00	90.74	4.54	5.07	1.29
Dry food 2	.	5.57	10.92	40.00	83.90	4.20	9.54	2.60
Dry food 3	.	5.91	9.05	36.00	88.49	4.42	8.14	2.05
Dry food 4	.	5.47	9.08	32.00	94.55	4.73	6.77	1.92
Dry food 5	.	5.73	8.02	30.00	92.61	4.63	6.48	1.73
Dry food 6	.	5.51	7.89	28.00	93.28	4.66	6.00	1.69
Dry food 7	.	5.64	6.03	22.00	92.93	4.65	4.73	1.30

Dry food 8	.	5.39	6.38	22.00	92.74	4.64	4.74	1.38
Dry food 9	.	5.16	10.54	34.00	83.64	4.18	8.13	2.52
Dry food 10	.	5.91	8.44	34.00	93.36	4.67	7.28	1.81
Homema de 1	.	6.79	2.49	12.00	21.76	1.09	11.03	2.29
Homema de 2	6.43	6.55	2.80	13.00	25.46	1.27	10.21	2.20
Homema de 3	6.12	6.37	2.28	10.00	26.15	1.31	7.65	1.74
Homema de 4	5.16	5.81	2.57	10.00	23.85	1.19	8.38	2.15
Homema de 5	6.14	6.35	2.28	10.00	30.41	1.52	6.58	1.50
Homema de 6	5.84	6.05	2.65	11.00	31.01	1.55	7.09	1.71
Homema de 7	5.97	6.24	2.57	11.00	29.04	1.45	7.58	1.77
Homema de 8	6.00	6.22	2.32	10.00	29.31	1.47	6.82	1.58
Homema de 9	6.11	6.40	2.21	10.00	19.19	0.96	10.42	2.31
Homema de 10	6,09	6,34	183	8.00	27.93	1.40	5.73	1.31