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The Phosphate Additives Phosphoric Acid and Sodium Phosphate Lead to Hyperphosphatemia as well as Increased FGF23 and Renal Phosphate Excretion in Healthy Cats

Carla Steffen and Britta Dobenecker*

Chair of Animal Nutrition and Dietetics, Department of Animal Sciences, Ludwig-Maximilians-Universität, Schönleutnerstr. 8, 85764 Oberschleissheim, Germany

***Corresponding author:** Britta Dobenecker, Chair of Animal Nutrition and Dietetics, Department of Animal Sciences, Ludwig-Maximilians-Universität, Schönleutnerstr. 8, 85764 Oberschleissheim, Germany

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Abstract

Chronic Kidney Disease (CKD) is an increasingly frequent disease in geriatric cats, representing the leading cause of death in this species. Reports about the increasing prevalence of CKD exist also in human medicine. The intake of nutrients, notably phosphorus, can affect renal health. Dietary supply with phosphate can influence concentrations of phosphate and factors of the phosphate homeostasis in blood and urine, possibly causing adverse health effects. Investigating nutritional factors involved in the cause and aggravation of kidney damage in more detail is of high importance not only for feline health. The cat is already successfully used as model for various human diseases which highlights its potential role as translational model for phosphate toxicity in humans. Effects of the food additives H_3PO_4 and NaH_2PO_4 on phosphorus homeostasis were investigated in comparison to a control diet. Adding these highly available phosphate additives to the diet of healthy cats for 28 days caused a significant increase in serum phosphate, calcium, and FGF23 as well as in renal phosphorus excretion. Consequently, the use of both phosphate additives in food processing needs to be critically re-evaluated due to the high availability of these sources and therefore their potential adverse health effects. With up to 80 % prevalence, Chronic Kidney Disease (CKD) is one of the most common chronic diseases in geriatric cats [1,2], and represents the leading cause of death in this species [3]. Reports of the increasing prevalence of CKD in the last decades also exist in human medicine [4]. It has been shown in various species, including humans and cats, that the supply of nutrients, notably phosphorus, can affect renal health [5-7]. To investigate nutritional factors involved in cause and aggravation of kidney damage in more detail is therefore of high importance not only for feline health. The fact that the cat has been successfully used as a model for human diseases in several studies [8-12] supports its potential role as translational model for human medicine.

Keywords: Chronic kidney disease; Food processing; Nutrition; Phosphate excess; Phosphorus homeostasis

Introduction

Elevated serum phosphate concentrations have been identified as major risk factor for progression of CKD and correlate strongly with mortality in humans [13] and cats [14-16], and independently predicts progression of CKD [17]. Therefore, stabilizing serum phosphate values by lowering phosphate intake, especially of inorganic phosphates, is a key element of dietary CKD management [18,19]. Apart from the relevance of inorganic phosphate intake in renal patients, dietary supply with inorganic phosphate can also cause adverse health effects in healthy individuals. In studies carried out in the 1930s, MacKay et al. observed permanent renal lesions in rats after feeding diets high in phosphoric acid (2.94 %) [20]. In dogs, oral administration of potassium phosphate (30 mg /kg $BW^{0.75}$) caused tubular atrophy within weeks [21]. Dobenecker et al. (2018) showed that high phosphate supply affected kidney function in cats after only 28 days of feeding calcium and sodium monophosphate [6]. In healthy male adults, FGF23 was significantly higher when supplementing the diet with inorganic phosphate [22] and was found to be positively correlated with renal phosphorus excretion [23]. Restriction of inorganic phosphate intake might therefore be important for treatment or even prevention of CKD and other adverse health effects linked to a high phosphate burden.

Dietary supply with phosphate, influenced by its amount and source, determines the phosphorus influx into the body. Studies investigating phosphorus kinetics showed that most inorganic phosphates, due to their high solubility in water, are absorbed in larger quantities compared to phosphates form organic sources [24-26]. Therefore, the phosphate burden cannot be assessed by the total phosphate intake alone, as its source needs to be considered as well. The clinical relevance of phosphate bioavailability has been demonstrated repeatedly in several species. In dogs, postprandial serum phosphate concentrations as well as parameters of phosphorus homeostasis such as PTH and FGF23 increased significantly after high oral phosphate intake from inorganic but not from organic sources [24]. In rats, nephrocalcinosis was more severe when feeding tripolyphosphates compared to dihydrogen phosphates [27]. Another study in mice found health effects of polyphosphates to be more harmful compared to monophosphates [28]. In cats, adding phosphate from inorganic sources (sodium or potassium phosphate) but not from an organic source (bone meal) led to a significant increase of renal phosphorus excretion [29]. Different effects of organic versus inorganic phosphates on phosphate homeostasis due to differences in availability and digestibility were also observed *in vitro* [30,31] as well as in humans [32]. Consequently, the source of dietary phosphate needs to be considered when formulating diets for CKD patients as well as healthy individuals. Yet, a recent study of Dobenecker (2021) [33] demonstrated that the concentration of phosphate in commercial pet foods, including renal diets, often greatly exceeds the recommended allowance given by the NRC [34] and these diets often contain high percentages of highly water soluble phosphate. A considerable number of products for human consumption are also known to be enriched with inorganic phosphate and are therefore not recommended for kidney patients [26,35-37]. Further research investigating effects of different sources of phosphate on health parameters is therefore warranted. The availability and range of appropriate parameters is key when researching the effects of different phosphate sources on mineral homeostasis and renal function. Relatively recently, Fibroblast Growth Factor-23 (FGF23) has been established as an early marker of disturbed phosphorus metabolism in human [38,39], feline [40,41] and canine [42] renal patients due to its crucial role in phosphorus homeostasis as phosphatonin [43]. Its most effective pathway involves the kidneys: as a response to phosphate influx into the bloodstream, FGF23 increases the urinary phosphorus excretion by degrading the sodium-phosphate cotransporter in the proximal tubules [44]. A high, potentially toxic serum phosphate concentration usually leads to an increase in serum FGF23 and renal phosphorus excretion [23,45,46]. This elimination strategy might cause consequences itself, because the degree of renal damage (tubular-interstitial fibrosis and tubular atrophy) correlates directly with the amount of phosphorus excreted per nephron [47]. In addition to the effect of FGF23 on the kidneys, further potentially harmful actions of its (chronically) increased concentrations have been identified on cardiovascular health [48,49], vitamin D metabolism and bone mineralisation [50,51].

Another parameter to assess potential damage on kidney health is the serum calcium by phosphorus product (sCaxP). Values of sCaxP above 55 mg^2/dl^2 are associated with soft tissue calcification in humans [52], and concentrations >70 mg^2/dl^2 are correlated with decreased life expectancy in canine renal patients [53] and interdigital calcifications in cats [54] with CKD. Dietary phosphate can derive from naturally occurring organic sources, mineral supplements or inorganic sources added during food processing. Due to their various properties, phosphate additives are also widely used in pet food. In cat food, phosphoric acid is applied as texturizer [37,55,56], urine acidifier (prevention of uroliths) [57,58], to enhance palatability especially in dry food

kibbles [59] and as preservative [60]. In the list of commonly used phosphate containing GRAS (generally recognized as safe) substances in food as well as pet food processing, phosphoric acid is noteworthy as a liquid phosphate source with an exceptionally high concentration of phosphorus (32 %) [61]. It is important to note that phosphate from phosphoric acid is described as almost completely absorbable [26,62]. In this source, phosphate appears in a completely dissolved and highly available form. For poultry feed, Kirstein et al (2017) reported that phosphate availability was the highest (84 %) when diets were supplemented with phosphoric acid compared to other sources of inorganic phosphate [63]. For beverages containing phosphoric acid, its bioavailability is reported to reach 100 % [64]. So far, data regarding effects of phosphoric acid on apparent digestibility, phosphorus and calcium homeostasis and possible adverse health effects are scarce, but the existing data raises concerns regarding the safety of phosphoric acid. Concerning effects of phosphoric acid intake have been observed in humans as well as in cats. In healthy young men, consumption of phosphoric acid containing beverages was found to increase serum phosphate concentrations and bone resorption [5]. In cats, Fettman et al. (1992) observed an increase in renal phosphorus excretion after supplementing the test diet with phosphoric acid [65]. In another study by DiBartola et al. (1993), 5/9 cats developed renal lesions over a course of 2 years on a commercial diet containing phosphoric acid [66].

Intake of soluble phosphate sources can cause an increase in serum phosphate concentrations, resulting in hyperphosphatemia [24,67-70]. One major pathway to maintain serum phosphate concentrations stable is to upregulate its renal excretion [23]. Compared to other species, this can lead to even higher urinary phosphorus concentrations in the cat because of its peculiarity in producing highly concentrated urine [71]. Based on the information that the amount of phosphorus per nephron correlates directly with the degree of renal damage [47], it can be hypothesized that the cat is especially sensible to an excessive intake of phosphorus which also explains the high prevalence of CKD in this species triggered by these two factors. In other words, the oral intake of highly available phosphates is presumably more critical in this species because of the physiologically high specific gravity of the urine [71]. Thus, the cat may be suitable as a translational model of phosphate toxicity for human medicine to study long-term effects in a time-lapse fashion. Therefore, the aim of this study was to investigate the effects of dietary supply of phosphoric acid (H_3PO_4) in combination with NaH_2PO_4, another highly available phosphate source [6,72-74], for 28 days on the phosphate and calcium balance as well as on serum phosphate and FGF23 in healthy cats also as potential model for human medicine.

Animals, Materials and Methods

The effects of an addition of inorganic phosphates from phosphoric acid combined with sodium phosphate on mineral balance and selected serum parameters were investigated in comparison to a control diet. Eleven (control group, CON) and ten (phosphoric acid + sodium phosphate, PA-NaP diet) healthy adult European shorthair cats (4 males, 7 females, 1-4 years of age, 2.7-4.7 kg body weight) bred and housed in the cattery of the chair of Animal Nutrition and Dietetics, Department of Veterinary Sciences, Ludwig-Maximilians-University Munich, participated in this study. All cats underwent a general health check directly before the start of the study including complete blood count and selected parameters of kidney function (urea, creatinine, SDMA, serum electrolytes) to ensure a proper health status. Each trial lasted 28 days, commencing with an adaptation phase (18 d), followed by a digestibility trial of 10 days. Quantitative sampling of urine and faeces was carried out, and food and water consumption were recorded. The trials were completed by taking fasted (minimum 12 hours (h) after the last meal) and postprandial (3 h after food intake) blood samples on day 28. Visual inspection of the cats was conducted daily by a veterinarian and a general health exam including weighing was performed on a weekly basis. The representative of the Veterinary Faculty for animal welfare and the Government of Upper Bavaria (reference number ROB 55.2-1-2532.Vet_02-19-38) approved the study.

Housing

During the adaptation phase cats were housed in groups of 4 to 8 animals. To allow individual food intake, the cats were separated into single cages during feeding times for a maximum of 1 h. The same cages for single housing to which the cats had been accustomed beforehand (length × width × height = 120 × 60 × 53 or 90 × 80 × 75 cm) were used during the digestibility trial. Air temperature and humidity were monitored and controlled using an air conditioning system. Light (natural and artificial) was available for at least 8 h a day. Fresh water was provided in stainless steel bowls ad libitum. The individual cages were equipped with seat boards, blankets and a litterbox.

Diets

A complete and balanced basal diet was produced for both parts of the trial and fed in two daily meals. Basic values of all parameters were determined for each individual during the control trial (Table 1). Food quantity was allocated based on historical data for individual energy requirements to maintain body weight. In

diet CON, phosphorus originated entirely from organic sources, meeting the recommended daily allowance for phosphorus (NRC 0.64 g/Mcal) [34]. Exclusive use of phosphoric acid to reach the targeted amount of inorganic phosphate was not possible due to palatability reasons. Therefore, sodium phosphate (NaH_2PO_4) was used to supplement phosphoric acid in the PA-NaP diet (Table 1). To adjust the pH between 6.8 and 7.2, potassium hydroxide (KOH) was added to the feed. In both diets, the Ca/P ratio was within the recommended range of 1/1 to 2/1.

Sample Collection and Storage

During the collection period, the cats were housed individually. To collect urine samples, two plastic basins were stacked on top of each other. The upper basin contained polyethylene beads as litter material to allow normal feline toileting behaviour. Slots in the bottom of the first basin allowed the fresh urine to pass through into the second basin. Urine was collected at several time points during the day with a maximum period in between of 12 h (overnight). Using thymol and paraffin is a reliable method to conserve the stability of urine pH over a period of at least 12 h, as demonstrated in in-house trials [75]. To ensure the reliability of the method, the pH of several fresh urine samples was measured and re-evaluated after 12 h of preservation. The amount of urine produced by each cat was determined by weighing. To collect the urine, the layer of paraffin-thymol-mixture was penetrated with a needle and aspirated. Urine pH (WTW pH 325, calibrated before measuring) and specific weight (refractometer HRM 18, Krüss Optronic, Germany) were determined directly after sampling. Daily samples were kept refrigerated, pooled, and stored at -18 °C thereafter. Faeces were collected quantitatively as soon as defecation was noticed; the samples were then weighed and stored at -18 °C until analysis. After freeze-drying (T 22 K-E-6, Piatkowsky, Munich, Germany), daily samples were pooled, ground and thoroughly mixed. Blood samples for serum and citrate plasma were drawn on day 28 preprandially (pre; at least 12 h fasted) and 3 h postprandially using either the vena saphena medialis or the vena cephalica antebrachii. After ~30 minutes and ~2 h (FGF23) of clotting, respectively, samples were centrifuged for 15 minutes at 2000 rpm (FGF23) and 10 minutes at 3000 rpm for the remaining parameters and stored at -80 °C until analysis. Wet digestion with 65 % HNO_3 was performed in a microwave system for feed and faecal samples. An aliquot of each 24 h urine sample was pooled after gentle thawing and proper stirring for analysis.

Laboratory Analyses

Calcium was analysed photometrically (flame photometry, Eppendorff EFOX 5033) in urine, faeces and serum. For analysis of phosphorus, the modified vanadate molybdate method modified according to Gericke und Kurmies (1952) [76] (Thermo-Spectronic, Genesys 10uv) was applied. Magnesium in the feed was measured via spectrometry (Perkin Elmer AAnalyst 800), chloride was analysed using a chloridometer (Slamed Chloridmeter 50µl), potassium and sodium were measured photometrically (flame photometry, Eppendorff EFOX 5033). Crude nutrients in the diets and faecal samples were determined by Weende analysis (VDLUFA 2012) [77]. Serum samples were analysed for FGF23 using an ELISA kit validated for feline samples (KAINOS Laboratories Inc., Tokyo, Japan) [40]. Serum creatinine was measured photometrically at IDEXX Vet Med Laboratories GmbH, Ludwigsburg, Germany, and urine creatinine was analysed in-house (MicroVue Creatinine Assay Kit, Quidel Corporation).

		CON	PA-NaP
Ingredients	%	Beef (Heart, Steak)	72
Basal diet		Rice	24
		Cellulose	1
		Rapeseed oil	3
GE	MJ/kg DM	27	
DM	g/kg	431	426
P source	mg/Mcal	698 (organic)	792 (organic) 485 (H_3PO_4) 1478 (NaH_2PO_4)
Crude protein	g/kg DM	439	497
Crude fat		362	325
Crude fibre		35	30
Crude ash		16	20
NfE		77	128
Ca		5	26
P		3.6	13.1
K		8	10
Mg		0.9	2.2
Na		1.4	6.7
Cl		6.1	2.0
Ca/P	-	1.3/1	2.0/1
Vit. D_3	IU/kg DM	449	382

DM: dry matter, GE: gross energy, NfE: nitrogen-free extract, Ca: calcium, P: phosphorus, K: potassium, Mg: magnesium, Na: sodium, Cl: chloride, Ca/P: Calcium to phosphorus ratio

Table 1: Composition of diets

Calculations and Statistical Analysis

Values between groups were compared with a Student's t-test while a paired-t-test was performed to compare pre- and postprandial values. To test for normality, a Shapiro-Wilk test was applied. Results with p-values ≤ 0.001, ≤ 0.01 and ≤ 0.05, respectively, were considered significantly different. Apparent digestibility (aD) during the 10day collection period was calculated as follows: aD [%] = (nutrient intake$_{feed}$ – nutrient excretion$_{faeces}$)/ nutrient intake$_{feed}$.

Results

Nutrient Intake and Balance

All cats stayed clinically healthy throughout the study. In the PA-NaP diet, water intake increased significantly compared to CON (43±9 ml/kg vs. 29±3 ml/kg BW/d; p ≤ 0.001). In alignment, urine volume increased as well (20±8 vs. 14±3 g/kg BW/d; p ≤ 0.022; Table 4). Intake of dry matter did not differ between groups (13±1 g/kg BW vs. 12±1 g/kg BW), but apparent digestibility of dry matter was about 10 % lower in the PA-NaP diet compared to CON (p ≤ 0.001; Table 2). While apparent digestibility of calcium remained unaffected by the diet, the apparent digestibility of phosphorus decreased significantly in the PA-NaP diet (p ≤ 0.001). Still, the amount of apparently digested phosphorus was significantly higher in the PA-NaP diet compared to CON (50 ± 16 vs. 29 ± 3 mg/kg BW/d, p ≤ 0.001). Renal phosphorus excretion increased significantly when inorganic phosphate was added to the diet (p ≤ 0.001). Despite a significantly increased concentration of phosphorus in the urine of cats fed the PA-NaP diet (p < 0.001; Table 4,3), retention of phosphorus did not differ between groups. Similarly, calcium concentrations were slightly but significantly higher in the PA-NaP diet compared to CON (p < 0.01), while calcium retention remained unaffected.

Mineral	Diet	Intake mg/ kg BW	Faecal ex. mg/kg BW	aD %	Renal ex. mg/kg BW	Retention mg/ kg BW	App. digest. mg/ kg BW
P	CON	49±3	19±4	60±9	14±5	15±4	29±3
	PA-NaP	157±20***	110±13***	30±8***	45±14***	2±18	50±16***
Ca	CON	64±4	58±11	10±17	0.4±0.1	6±11	6±11
	PA-NaP	321±42***	311±37***	3±9	0.8±0.3***	9±31	10±31

ex.: excretion, aD: apparent digestibility, App. digest.: apparently digested, Ca: calcium, P: phosphorus,

*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 between groups

Table 2: Intake, renal and faecal excretion, retention, and apparent digestibility of phosphorus and calcium (mean ± standard deviation)

Blood Parameters

Postprandial FGF23 serum concentrations were lower compared to the fasted state in both groups, but only significantly in CON (p = 0.001). Compared to CON, the PA-NaP diet led to a highly significant increase of serum FGF23 concentrations at both time points (p ≤ 0.001). Phosphorus intake from organic sources in CON resulted in significantly lower serum phosphate values postprandially. In contrast, the inorganic phosphate in in the PA-NaP diet caused significantly lower preprandial serum phosphate values compared to CON (p = 0.019), which then significantly increased by 50 % after food intake (p ≤ 0.001). In 8/10 cats, postprandial serum phosphate concentrations exceeded the upper reference range. In the PA-NaP diet, serum calcium values were significantly higher before and after feeding compared to CON (p ≤ 0.001). Within groups, fasting values for calcium were significantly lower in the PA-NaP diet as well as CON (p = 0.018; p = 0.013). Accordingly, the preprandial serum calcium by phosphorus product (sCaxP) did not differ between groups. Due to the postprandially increased calcium (p ≤ 0.001) and phosphate serum concentrations in the PA-NaP diet, values for sCaxP rose above the threshold of 55 mg/dl given by Block et al. (2000) [52] in all cats. In contrast, a significant decrease of sCaxP values 3 h after food feeding (p ≤ 0.001) was seen in the CON group. Serum creatinine concentrations increased after food intake in both groups (CON: p = 0.007; PA-NaP: p = 0.033) and were significantly higher postprandially in the PA-NaP diet compared to CON (p ≤ 0.001).

Serum	Time point	Reference range	CON	n outside reference range	PA-NaP	n > reference range
FGF23	pre	< 300	202±53	1/11	354±79***	8/10
[pg/ml]	post		142±22#	0/7	282±80***	3/10
P	pre	0.8-2.2	1.8±0.2	2/11	1.6±0.1*	0/10
[mmol/l]	post		1.4±0.1#	0/11	2.4±0.2#***	8/10
Ca	pre	2.2-2.9	2.3±0.1	0/11	2.6±0.1***	0/10
[mmol/l]	post		2.2±0.0#	2/10	2.4±0.1#***	0/10
sCaxP	pre	< 55·	52±6	2/11	51±4	0/10
[mg²/dl²]	post		39±3#	0/10	71±5#***	10/10
Creatinine	pre	0.08-0.20	0.14±0.01	0/11	0.15±0.01	0/10
[mmol/l]	post		0.16±0.01#	0/11	0.18±0.01#***	0/10

Ca: calcium, P: phosphorus, sCaxP: serum calcium by phosphorus product, pre: preprandial, post: postprandial

*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 between groups; #pre- and postprandial values within one group differ significantly (p ≤ 0.05); • Block et al. (2000) [52]

Table 3: Serum parameters after 28 days of either high phosphate or control feeding in cats (mean ± standard deviation)

Urine Parameters

All urine parameters were significantly affected by high H_3PO_4 and NaH_2PO_4 intake. Urine creatinine concentrations were lower in the PA-NaP diet (p ≤ 0.001), while phosphorus (p ≤ 0.001) concentrations were significantly higher despite the increase in urine volume (p ≤ 0.022) and the decrease in urine specific gravity (p ≤ 0.001). The same applies to the urinary calcium concentrations in the PA-NaP diet (p = 0.002), but because all values were below the validated detection limit for calcium in the photometric analysis, the validity is restricted.

Urine	CON	PA-NaP	Reference range
P [g/l]	1.0±0.2	2.4±0.6***	-
Ca° [g/l]	< 0.04	< 0.04	-
Creatinine [mmol/l]	32±4	21±4***	-
USG [mg/ml]	1060±2	1045±7***	1035-1060
Volume [ml/kg BW/d]	14±3	20±8**	< 50

USG: urine specific gravity, Na: sodium, Cl: chloride, K: potassium, P: phosphorus, P/Crea = phosphorus to creatinine ratio; ° all measured values below given detection limit; *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 between groups

Table 4: Urine parameters after 28 days of either high phosphate or control feeding in cats (mean ± standard deviation)

Discussion

CKD is the most common terminal disease in aging cats [3]. In humans, the prevalence of CKD is also high and increasing [4]. Phosphate is known to be a main contributor to the progression of CKD [13-17]. Demonstrated adverse health effects from oral ingestion of highly available phosphates such as phosphoric acid [65,66] have raised concerns about the safety of additives even in healthy individuals. Research in felines can help understand factors that promote kidney damage also in human patients. Moreover, inorganic sources of phosphate are used in cat food as well as human food and beverages. Understanding the impact of different sources of phosphate on health parameters is therefore important. Although phosphoric acid is used, for example, as acidulant, preservative, or palatant in pet food [57-60], empirical studies investigating its safety are still scarce. Therefore, this study aimed to gain further insights regarding the effects of phosphoric acid in combination with another source of highly available inorganic phosphate, both with relevance for feline and potentially human nutrition and health.

In order to avoid an inverse calcium to phosphorus ratio, which in itself might cause effects on the phosphorus availability, and to better reflect the pet food market, the calcium supply was increased to achieve a ratio between 1 and 2 to 1. Separate effects of calcium on parameters such as FGF23, described in genetically modified mice [78], were not expected in the own trial. It is known from practical feeding as well as preceding trials, that a nutritional supply with calcium in this magnitude does not lead to hypercalcemia in cats and dogs [7,24,70,79] nor to increased FGF23 concentrations in case of excessive calcium supply from 'organic' sources like meat and bone meal [24,70]. Furthermore, the addition of calcium carbonate caused a slight but biologically probably irrelevant increase in serum calcium concentrations with all values within the reference range. The sodium intake in the test diet was increased by adding sodium phosphate up to levels which are comparable to processed commercial cat food. Besides, phosphate salts permitted as food and feed additives deliver a cation with the phosphate, in this case sodium. The majority of commercially available processed food products for cats supply major minerals in abundance [33,80,81], which makes the mineral profile of this test diet practically relevant.

Notably, there were significant differences between the groups in various parameters even after the short duration of each trial phase. One of the main findings was the significant increase of serum FGF23 after feeding the PA-NaP diet, independent of the sampling time. Dobenecker et al. (2021) [24] found comparable results when examining the kinetics and effects of inorganic versus organic phosphates added to the diet of dogs. Increased FGF23 concentrations, partly mediated by the co-factor klotho, are linked to several pathological findings. These are left ventricular hypertrophy [82,83], progression of renal failure and cardiovascular diseases through endothelial dysfunction, vascular stiffness and vascular calcification [84,85], cytokine activation leading to chronic inflammation [86,87], an increased risk of infection [88], heart failure [83], and reduced synthesis of active vitamin D [50] in CKD patients. Furthermore, elevated serum FGF23 concentrations might lead to FGF-receptor resistance, further exacerbating the adverse effects mentioned [89]. Whether the association between FGF23 and clinical events is causal, or casual remains controversial [90] and extensive further research is needed to answer this question. However, as long as it is not fully understood to what extent FGF23 contributes to disease rather than merely indicating it, the use of additives causing a significant prolonged increase should be avoided.

Despite the considerable increase of FGF23, the mean postprandial serum phosphate concentrations exceeded the upper reference value in most cats. This might indicate that the regulation by FGF23 was delayed or even insufficient to normalize serum phosphate 3 h postprandially due to the high influx of inorganic phosphate into the bloodstream. In dogs fed 1 meal per day, peak values for serum phosphate concentrations were found 3 h after food intake and had not returned to normal after 7h [24]. In another study in dogs, the addition of inorganic phosphates to the diet also caused a significant postprandial increase of serum phosphate and, in the case of monosodium and monopotassium phosphate, a significant decrease in preprandial samples [70]. In contrast, high phosphate intake from organic sources (bone meal) did not affect serum phosphate values in the respected trials [24,70]. Therefore, the observed increase in serum phosphate values can be linked to the application of inorganic phosphate.

Data in the current study support this finding, where serum phosphate in cats were significantly lower after fasting for at least 12 h compared to postprandial concentrations when fed the high phosphate diet. Presumably, the persistent elevation of serum FGF23 with significantly higher values also preprandially caused a drop of serum phosphate below the values measured in the control group. This finding has practical implications, as routine diagnostics usually include measurement of fasted serum phosphate concentrations, but not of postprandial values. Our study clearly showed that only postprandial serum phosphate concentrations rose above the upper reference, while serum FGF23 values remained elevated in the PA-NaP diet compared to CON.

Evaluating postprandial serum phosphate values might therefore be useful when assessing phosphorus homeostasis, due to possible circadian and dietary influences [91].

In the PA-NaP diet, the apparent digestibility of phosphorus was half of what was measured in the control group. However, the apparently digested amount of phosphorus was significantly higher in the PA-NaP diet ($p<0.001$), explaining the increase of postprandial serum phosphate and renal phosphorus excretion. Considering the apparent digestibility of phosphorus alone after supplying different sources of phosphate can therefore be misleading when assessing the impact of different dietary phosphate sources. The fivefold increase of calcium supply in the PA-NaP diet did not affect the apparent digestibility or the apparently digested amount of this element. Most of the additional calcium was excreted faecally, as expected [92]. Even though the renal calcium excretion proofed to be statistically higher in the PA-NaP diet, the amount per kg BW was too low to be biologically relevant. Regarding serum calcium concentrations, interesting results were found as well. In contrast to findings reported by Geddes and coworkers [93] in feline CKD patients after their dietary change to a phosphorus restricted diet, the lower phosphorus intake in the CON group did not cause an increase in serum calcium. To the contrary, the higher phosphorus concentration in the PA-NaP diet lead to significantly higher pre- and postprandial serum calcium concentrations, presumably also because of the higher calcium intake in this group. The authors of the aforementioned paper did not mention if maybe calcium-containing phosphate binders were used in this group of cats with diagnosed CKD, which would explain the observed trend to higher serum calcium [93].

Due to the increased serum calcium and phosphate concentrations in the cats of the PA-NaP diet, mean postprandial sCaxP was significantly higher when compared to CON. Increased sCaxP values correlate with adverse health effects such as soft tissue calcification [52,54] and shortened life expectancy [53] in humans and non-human animals. Therefore, finding values above the threshold of 55 mg^2/dl^2 [52] in all of the healthy cats in our study is alarming. Consequently, potential effects of food additives such as phosphoric acid on sCaxP should also be investigated in humans. Effects of sodium phosphate alone versus a combination of phosphoric acid and sodium phosphate on serum phosphorus and calcium and therefore sCaxP differ significantly in cats. The effect of phosphoric acid addition was significantly more pronounced compared to sole use of sodium phosphate, even though the total phosphate supply was higher in the trial using only sodium phosphate (4.5 g/Mcal) [94]. This is further proof that both the total amount of ingested phosphate and its source are responsible for the effects on calcium and phosphorus metabolism. Previously, a SUL for inorganic phosphate intake of 1 g/Mcal in cats has been suggested [95,96]. This recommendation was based on results from medium-term studies in cats using primarily sodium tripolyphosphate (STPP). In consideration of the present study results, more extensive information is needed about possible effects of all inorganic phosphate sources deployed in food processing, in combination with other dietary and individual factors, before postulation of any safe upper limit guaranteeing that inorganic phosphate additives are unconditionally safe for human and animal consumption.

Another factor to consider when interpreting research data using a translational approach is the choice of the animal model. The suitability of a research model is determined by various factors such as availability, maintenance costs, but also the degree of comparability of physiological function of the target organ or structure and, in case of nutritional context, the nutrition or food spectrum of both the animal model and the target species. A considerable percentage of research in the field of dietary impact on renal function has been carried out in rodents [97]. It is important to note that rodents do not fulfil all the factors mentioned above. Commonly used rodent-models such as the $5/6^{th}$ nephrectomy rat model [98,99] and the adenine nephrotoxic model [100,101] develop late stages of CKD due to acute kidney injury. Yet, progression of CKD in humans is usually slow, which limits the comparability of the pathophysiology [102]. Differences are also found in phosphorus absorption and vitamin D and bone metabolism, and while serum vitamin D values decrease in humans, they remain constant or even increase in mice as CKD progresses [103]. Additionally, Lindström et al. (2018) identified significant differences in the timing, scale, organization, and molecular profile of key cell types and composite cell structures between mice and humans [104]. The cat, on the other side, might have some advantages in comparison. Previous studies in healthy felines [6,7] using calcium and sodium monophosphate as sources of inorganic phosphate, found an increase in renal phosphorus excretion, glucosuria and a decrease in creatinine clearance, indicating potential renal damage [105]. It can be hypothesised that increased renal phosphorus excretion with high urine concentrations after excessive oral phosphate loading can be seen as cause for the conspicuously high prevalence [1,2] of CKD in this species. Due to the high sensitivity of the cat to orally administered phosphates, the cat might be a valuable model for human CKD research.

Conclusion

CKD has a high prevalence in humans as well as felines. Highly available sources of inorganic phosphate are known to adversely affect kidney health. Evaluating the safety of commonly used food additives such as phosphoric acid is therefore important. Adding phosphoric acid in combination with a soluble sodium phosphate to the diet of healthy cats caused a significant increase of serum FGF23, phosphate concentrations, and the serum calcium by phosphorus product as well as renal phosphorus excretion. In this study, we proposed the cat as translational model for human medicine, due to its high sensitivity to dietary phosphate. These findings raise serious concerns regarding the safety of using highly available phosphate sources in processing of food products for feline and human consumption.

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