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The Dietary Cation Anion Balance Exacerbates the Effects of Inorganic Phosphates on Parameters of Phosphate Metabolism in Cats

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Abstract

Dietary intake of inorganic phosphates is linked to various adverse health effects. Excessive intake of highly soluble inorganic phosphates, which are used as feed and food additives, have been found to impair parameters of kidney health. As chronic kidney disease represents one of the most frequently occurring terminal diseases especially in cats, extensive knowledge regarding the safety of these additives is important. Other minerals, such as calcium, can modulate their effects on the phosphate homeostasis and kidney health. Therefore, it is crucial to examine further factors, such as the dietary cation-anion balance (CAB), resulting from the concentrations of major minerals in a diet. In this study, eleven healthy cats were fed a control diet and two diets with added sodium monophosphate (NaH_2PO_4) with either a low (-10 mmol/kg dry matter) or high (+450 mmol/kg dry matter) CAB for 28 days each. The serum concentrations of phosphate and parameters of phosphate homeostasis were determined in the fasting and postprandial blood samples next to the apparent digestibility and retention of phosphate and calcium. The diet with positive CAB led to an increase of serum phosphate and the phosphatonin FGF23, apparently digested phosphate, and phosphate retention. This is further proof that source and amount of phosphates in a diet are not the only determinants of the extent of potential adverse health effects. Until the interactions between inorganic phosphates and other dietary compounds are fully understood, recommendations regarding the safe use of phosphate containing additives in pet food are precarious.

Introduction

Based on the amount and source of dietary phosphate as well as time of exposure, phosphate containing food and feed additives, i.e. inorganic phosphates, can cause adverse health effects, especially on kidney function, in humans [1-3] and animals [4,5,6-10]. Sodium monophosphate (NaH_2PO_4) supplementation caused a decrease in creatinine clearance and increased phosphaturia in cats [6, 11]. In pigs, inorganic phosphate intake resulted in tubular calcification, inflammation, fibrosis, glomerular degeneration and atrophy [12] while in dogs, this led to a disruption of calcium and phosphate homeostasis including an increase of parathyroid hormone (PTH) and fibroblast-growth-factor 23 (FGF23) [7]. The intensity of these effects

are influenced by the solubility and therefore availability of the phosphate source [7, 13, 14], which then effects phosphate homeostasis and renal phosphate excretion. As demonstrated in rodents, the amount of excreted phosphorus correlates directly with renal damage [15], for which reason the dietary supply is of utmost importance, especially because phosphate containing additives are widely used in processed pet food due to their diverse properties (e.g., water binding, preservation, palatability enhancement, dental calculus prophylaxis, and uroliths prevention via urine pH adjustment [16-20]). Additional factors influencing the effects of added inorganic phosphate on the body have to be taken into account: high calcium concentrations and a wide calcium to phosphorus ratio (Ca/P ratio), for example, are expected to lower the apparent digestibility of phosphate and vice versa [6, 21, 22]. However, as demonstrated in dogs, this correlation does not seem to apply to the same extend to highly soluble phosphate sources [23]. Further major minerals including their electric charge are also of interest in this context. They influence the bodies' cation anion balance (CAB), mineral balances [24, 25], and urine pH [24-29]. Various medical reasons require modification of dietary CAB in order to influence blood and urine pH [24, 30] .

The aim of this study was therefore to examine the influence of different dietary CAB on the apparent digestibility and renal excretion of phosphate as well as on further parameters of phosphate homeostasis in cats fed additional inorganic phosphate (NaH_2PO_4).

Animals, Materials and Methods

Eleven 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, LMU Munich, participated in a total of three feeding trials (control group, CON; negative CAB, nCAB; positive CAB, pCAB). Each cat underwent a general health check including complete blood and measurement of selected parameters of kidney function (urea, creatinine, SDMA, serum electrolytes, urinalysis) before commencing the study. The diet period of 28 days (d) consisted of an adaption (18 d) and a digestibility phase (10 d). During the digestibility phase, urine and faecal samples were collected quantitatively and food and water intake were measured. Blood samples were drawn on day 28 preprandially (at least 12 hours (h) fasted) and postprandially (3 h after food intake). Cats were examined visually on a daily basis by a veterinarian and underwent a weekly general clinical exam and weighing. Proceedings and protocols were in alignment with the guidelines of the Protection of Animals Act and approved by the representative of the Veterinary Faculty for animal welfare, as well as the Government of Upper Bavaria (reference number ROB 55.2.-2532.Vet_02-19-38).

Housing

All cats were kept in groups of 4-8 during the 18 d of the adaption phase. During the digestibility phase, cats were housed individually in cages (length × width × height = 120 × 60 × 53 or 90 × 80 × 75 cm) to which they had been accustomed before. Each kennel was enriched with elevated seat boards, blankets and/or towels as well as a litterbox. Fresh water was provided in stainless steel bowls ad libitum. Air temperature and humidity were controlled by an air conditioning system.

Diets

In CON, the cats were fed a complete and balanced diet without addition of inorganic phosphate to establish basic values. In diet nCAB and pCAB, sodium dihydrogen phosphate dihydrate (NaH_2PO_4+$2H_2O$) and calcium carbonate ($CaCO_3$) were added to each diet (table 1), resulting in a ratio of 25 to 75% organic to inorganic phosphate in both CAB diets. To achieve a negative CAB of -10 mmol/kg dry matter (DM) in diet nCAB, the basic diet was supplemented with methionine and $CaCO_3$

Table 1. Composition of diets

Composition of diets		CON	nCAB	pCAB
Ingredients basal diet	%	72 Beef (heart, steak) 24 Rice 1 Cellulose 3 Rapeseed oil		
Ca source	mg/Mcal ME	931 ($CaCO_3$)	2498 ($CaCO_3$) 1873 ($CaCl_2$)	4460 ($CaCO_3$)
P source		698 (organic)	794 (organic) 2388 (NaH_2PO_4)	
Na source		NaCl	NaH_2PO_4	NaH_2PO_4
DM	g/kg	431	409	409
GE	Kcal/kg DM	6449	6449	6449
ME	Kcal/kg DM	5176	4807	4793
crude protein	g/kg DM	439	466	466
crude fat		362	342	342
crude fibre		35	26	26
crude ash		16	20	20
NfE		77	146	146
Ca		4.7	21	21
P		3.6	15	15
K		8.2	6.5	6.2
Mg		0.9	1.1	1.4
Na		1.4	9.1	8.6
Cl		6.1	16	1.8
Ca/P		1.3/1	1.4/1	1.4/1
Vit. D_3	IU/kg DM	449	265	261
CAB	mmol/kg DM	-60	-10	+450

DM = dry matter, GE = gross energy, NfE = nitrogen-free extracts,
Ca = calcium, P = phosphorus, K = potassium, Mg = magnesium, Na = sodium, Cl = chloride,
Ca/P = calcium/phosphorus ratio, CAB = cation-anion balance

was partly exchanged with calcium chloride ($CaCl_2$). The daily amount of feed was apportioned individually based on the nutritional requirements according to the National Research Council (NRC) and the European Petfood Industry (FEDIAF) to allow for maintenance of individual body weight. The mineral and vitamin supplement was not part of a complete diet but added separately to each individual. This was done in all three diets to ensure an identical mineral and vitamin supply per kg

bodyweight, independent of the individual energy requirements and therefore dry matter intake of each cat.

Sample collection and storage

Single housing during the digestibility phase allowed for individual collection of urine samples. A double layer-toilet system was used to achieve optimal compliance and to minimise environmental influences. To allow for normal feline behaviour while using the litterbox, the first basin was filled with inert polyethylene beads as litter material. Fresh urine passed through slots in the bottom and accumulated in the second basin, which was prepped with a mixture of thymol and paraffin to preserve the urine until collection. As shown in in-house investigations, this method reliably ensures a stable urinary pH over a period of at least 12 h [14]. Additionally, the pH values of fresh urine samples were compared with preserved samples to ensure reliability of the method. The amount of urine was measured by weighing. The urine was extracted by penetrating the surface of the paraffin-thymol -mixture and aspirated with a needle. Directly after sample collection, pH (WTW pH 325, calibrated before measuring) and specific weight (refractometer HRM 18, Krüss Optronic, Germany) were measured. Daily samples were kept refrigerated until they were pooled per day and stored at -18 °C. After gentle thawing, an aliquot of each 24 h sample was used to measure potassium (K), sodium (Na), calcium (Ca), phosphate (P), magnesium (Mg), chloride (Cl), and creatinine (Crea). Faeces were collected as soon as defecation was noticed, then weighed and stored at -18°C until analysis. After freeze-drying (T 22 K – E- 6, Piatkowsky, Munich, Germany), daily samples were pooled and ground.

Blood for serum was drawn preprandially (pre; at least 12 h fasted) and 3 h postprandially (ppr), from either the V. saphena medialis or the V. cephalica antebrachii to allow for measurement of P, total Ca, and FGF23. After allowing the blood to clot for ~30 minutes, samples were centrifuged at 3000 rpm for 10 minutes. Samples used to measure FGF23 were set aside to clot for ~2h and centrifuged at 2000 rpm for 15 minutes. Until analysis, all samples were stored at -80°C.

Laboratory analyses

Wet digestion with 65 % HNO_3 was performed in a microwave system for feed and faecal samples. The modified vanadate molybdate method according to Gericke und Kurmies (1952) [31] (Thermo-Spectronic, Genesys 10uv) was used to measure phosphate content in urine, faeces and feed samples. Crude nutrients in the diets and faecal samples were determined by Weende analysis (VDLUFA 2012) [32]. Flame photometry (Eppendorf EFOX 5053) was performed to analyse calcium, potassium and sodium. Magnesium was measured using spectometry (Perkin Elmer AAnalyst 800) and chloride was analyzed via a chloridometer (Slamed Chloridmeter 50µl). An ELISA kit validated for feline samples (KAINOS Laboratories Inc., Tokyo, Japan)[33] was used to analyse for serum FGF23. 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).

Calculations and statistical analysis

Results between the groups were compared via one-way ANOVA. A paired-t-test was performed to compare pre- and postprandial values. To test for normality, a Shapiro-Wilk test was applied, and equality of variance was tested with the Brown-Forsythe test. Normally distributed data was compared between groups using the Holm-Sidak test. In case testing for normality or homogeneity of variance failed, a Kruskall-Wallis One Way Analysis of Variance on Ranks was initiated. Results with p-values ≤ 0.05, were considered significantly different. Apparent digestibility (aD) during the 10 d collection

period was calculated as follows: aD [%] = (nutrient $intake_{feed}$ – nutrient $excretion_{faeces}$)/nutrient $intake_{feed}$ x 100. The retention of phosphorus and calcium was calculated as: retention = intake – faecal excretion – renal excretion.

The CAB of the diets were calculated as:

CAB [mmol/kg DM] = 49.9*Ca + 82.3*Mg + 43.5*Na + 25.6*K - 64.6*P - 13.4*met - 16.6*cys - 28.2*C1

(Mg: magnesium, Na: sodium, K: potassium, met: methionine, cys: cystine, Cl: chloride)

Results

All cats remained clinically healthy throughout the study. Water intake and urine volume did not differ between groups. DM and ME intake decreased in the high phosphate (CAB) diets (nCAB: 10 ± 1; pCAB: 11 ± 1 g/kg BW/d) compared to CON (13 ± 1 g/kg BW/d; $p > 0.001$ and 0.003) and along with it, faecal excretion of DM (1.3 ± 0.3 vs. 2.0 ± 0.3 vs. 2.0 ± 0.3 g/mg/kg BW; $p < 0.001$). Apparent digestibility of DM did not differ between groups (CON: 90 ± 2; nCAB: 92 ± 1; pCAB: 91 ± 2 %). Apparent digestibility of phosphorus was significantly lower in diet nCAB compared to CON and pCAB ($p < 0.001$ and $p = 0.017$; table 2). Compared to diet pCAB, the apparently digested amount of phosphorus was lower in diet CON ($p < 0.001$) and diet nCAB ($p = 0.019$) (table 2). The phosphate retention was also significantly higher in diet pCAB compared to nCAB ($p = 0.015$).

The apparently digested (CON: $p < 0.001$; nCAB: $p = 0.019$) and retained (CON: $p = 0.04$; nCAB: $p = 0.015$) amount of phosphorus was highest in the pCAB group (table 2). Irrespective of the CAB, urine phosphorus concentrations ([g/l]; table 4) as well as renal phosphorus excretion [mg/kg BW] was higher in both CAB groups compared to CON ($p < 0.001$). Even though the apparent calcium digestibility was not statistically different ($p = 0.08$), the apparently digested calcium was significantly reduced in diet pCAB ($p = 0.04$). In this group, the calcium retention was negative and significantly lower compared to CON ($p = 0.04$) while renal excretion of calcium did not differ between groups

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

Mineral	Diet	Intake mg/kg BW	Faecal ex. mg/kg BW	aD %	app. digested mg/kg BW	Renal ex. mg/kg BW	Retention mg/kg BW
P	CON	49±3^{a}	19±4^{a}	60±9^{a}	29±3^{a}	14±5^{a}	15±4^{ab}
	nCAB	173±22^{b}	132±18^{b}	24±7^{b}	41±13^{a}	33±8^{b}	8±14^{b}
	pCAB	182±24^{b}	120±15^{b}	34±5^{c}	62±14^{b}	36±10^{b}	26±15^{a}
Ca	CON	64±4^{a}	58±11^{a}	10±17	6±11^{a}	0.4±0.1	6±11^{a}
	nCAB	242±30^{b}	241±33^{b}	0±6	1±13^{ab}	0.3±0.1	1±13^{ab}
	pCAB	256±33^{b}	267±36^{b}	-5±9	-12±22^{b}	0.4±0.1	-12±22^{b}

ex. = excretion, aD = apparent digestibility, app. digest. = apparently digested, Ca = calcium, P = phosphorus

Columns not sharing a superscript letter are significantly different ($p < 0.05$)

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

Serum	Time point	CON	n > reference range	nCAB	n > reference range	pCAB	n > reference range	Reference range
FGF23 [pg/ml]	pre	202±53[a]	1/11	222±48[ab]	1/11	320±158[b]	3/10°	< 300
	post	142±22[#a]	0/7°	173±51[#ab]	0/11	264±182[b]	3/10°	
P [mmol/l]	pre	1.8±0.2[a]	2/11	1.5±0.1[b]	0/11	1.6±0.1[b]	0/11	0.8-2.2
	post	1.4±0.1[#a]	0/11	1.6±0.2[ab]	0/11	2.0±0.3[#b]	5/11	
Ca [mmol/l]	pre	2.3±0.1	0/11	2.4±0.2	0/11	2.5±0.2	0/11	2.2-2.9
	post	2.2±0.0[#a]	2/10°	2.4±0.2[b]	0/11	2.6±0.2[b]	0/11	
sCaxP [mg^2/dl^2]	pre	52±6[a]	3/11	45±4[b]	0/11	54±10[b]	4/11	< 55•
	post	39±3[#a]	0/10°	49±5[b]	3/11	64±10[#b]	0/11	
Crea [mmol/l]	pre	0.14±0.01	0/11	0.14±0.02	0/11	0.14±0.02	0/11	0.08-0.2
	post	0.16±0.01[#]	0/11	0.14±0.02	0/11	0.15±0.02	0/11	

FGF23 = fibroblast-growth factor 23, Ca = calcium, P = phosphorus, sCaxP = serum calcium by phosphorus product, Crea = creatinine

pre: preprandial, post: postprandial

[#]pre- and postprandial values within one group differ significantly ($p < 0.05$)

Columns not sharing a superscript letter are significantly different ($p < 0.05$)

°less than 11 samples measured due to insufficient amount of sampling material

•Block et al. (2000) [34]

($p = 0.31$; table 2).

Blood parameters

The amount of serum did not suffice to measure Ca and FGF23 in one and 4 cases, respectively, in diet CON, and FGF23 in another case in diet pCAB (table 3). Serum FGF23 decreased postprandially, a difference statistically significant in CON ($p = 0.028$) and nCAB ($p = 0.035$). Compared to CON, pre- and postprandial serum FGF23 concentrations were significantly higher in diet pCAB ($p = 0.028$). Preprandial serum phosphate concentrations were lower in both test diets compared to CON (nCAB: $p < 0.001$; pCAB: $p = 0.003$), while postprandial values increased significantly in pCAB ($p = 0.002$), exceeded the upper reference in 5/11 cats and were higher than in CON ($p < 0.001$). In contrast, serum phosphate concentrations decreased in CON after food intake ($p < 0.001$). Preprandial serum calcium concentrations did not differ between groups, while in CON postprandial values decreased and were significantly lower than in both CAB groups (nCAB: $p = 0.26$; pCAB: $p < 0.001$). In alignment, the serum calcium by phosphorus product (sCaxP) was significantly higher preprandially in group pCAB ($p = 0.017$) and postprandially in both CAB diets ($p < 0.001$; $p = 0.037$) when compared to CON. In diet pCAB, in 9/11 cats the threshold of 55 mg^2/dl^2 serum, given by Block et al. (2000)[34], was exceeded. The significant postprandial increase of serum phosphate values led to a significant increase of sCaxP in pCAB ($p = 0.027$) while this parameter decreased in CON ($p < 0.001$) parallel to the

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

Urine	CON	nCAB	pCAB	Reference range
P [g/l]	1.0±0.2[a]	2.5±0.4[b]	2.5±0.4[b]	-
Creatinine [mmol/l]	32±4[a]	30±5[ab]	26±4[b]	-
P/Crea	1.0±0.3[a]	2.8±0.3[b]	3.1±0.5[b]	-
USG [mg/ml]	1060±2[a]	1058±5[b]	1055±6[c]	1035-1060
Volume [ml/kg BW/d]	14±3	14±5	15±6	< 50•

P = phosphorus, USG = urine specific gravity

Ca: all measured values below detection limit of 0.04 g/l

•NRC (2006) [57]

Columns not sharing a superscript letter are significantly different ($p < 0.05$)

postprandial decrease in serum phosphate. Serum creatinine concentrations were not influenced by diet and increased postprandially only in CON ($p = 0.007$).

Urine parameters

In line with water intake, urine volume was not affected by diet, while urine specific gravity (USG) differed between all groups ($p \leq 0.001$). Urine creatinine values were lowest in diet pCAB but differed only from CON ($p < 0.001$). Phosphate concentrations in the urine increased in both CAB diets compared to CON (nCAB: $p = 0.004$; pCAB: $p = 0.005$; table 4). All Ca values measured in the urine were below the detection limit.

Discussion

To date, knowledge about the effects of source and amount of other dietary minerals, or the combination thereof, on the consequences of inorganic phosphate intake is limited. Considering the exceptionally high prevalence of CKD in felines [35] and the established effects of inorganic phosphate intake on renal health, the aim of this research was to investigate the dietary CAB as a potential factor of influence on availability and selected effects of dietary inorganic phosphate. This is especially important in feline nutrition due to the variability of the CAB in cat food, for example in diets intended to influence urinary pH and therefore urolith formation. As struvite uroliths are soluble in acidic solutions [36], commercial diets commonly aim for a slightly acidic to neutral urine pH to prevent struvite formation [26, 37, 38], while medical diets for struvite dissolution aim for a urine pH between 5.9 - 6.4 [29]. Because urine pH and CAB of a diet correlate closely, calculating the CAB allows the prediction of the average daily urine pH which in turn can be altered by adjusting the concentration of minerals in a diet [30]. A CAB between -60 and +100 mmol/kg DM approximately results in urine pH values between 6.0-7.0 [39]. Accordingly, diets with a CAB ≤ 0 mmol/kg DM have been proven effective in the treatment and prevention of frequently occurring uroliths in dogs and cats and are therefore often prescribed [40, 41]. The CAB of diet pCAB, however, results in the relatively high predicted urine pH value of 7.7.

In the current investigation, which was done applying a well-established study design [7, 8, 11, 13, 42, 43], the effects of feeding diets with added inorganic phosphate of an identical source and the same Ca/P ratio

but different CAB due to the use of varying calcium sources ($CaCO_3$, $CaCl_2$) and methionine addition were investigated in a short term study. Calcium carbonate has an alkalizing effect while calcium chloride reduces CAB and urine pH [30]. The use of these two calcium sources was necessary to adjust the CAB in the test diets with the lowest possible effect on the concentrations of other minerals. Whether the calcium source itself had a separate effect on the parameters measured in this study cannot be determined based on the current results. In addition, this research was conducted in a group of relatively young cats. Younger individuals were purposely selected to reduce the likelihood of early stage kidney disease despite normal blood test results. Because energy and nutrients were apportioned based on the individual metabolic body weight of each cat, the nutrient supply per unit of body weight was consistent. Attention was also paid to a balanced gender distribution and an ideal body condition. Hence, the chosen test group is representative of a healthy adult cat population.

Adding inorganic phosphate in the form of sodium phosphate to a balanced control diet containing solely organic phosphates caused a significantly altered phosphate balance in both test diets. Beyond this, a positive CAB of + 450 mmol/kg DM led to a significantly higher apparent digestibility and retention of phosphate: compared to the diet nCAB with a negative CAB of -10 mmol/kg DM, the amount of apparently digested phosphate per kg BW was about 50 % higher and differed also significantly from CON. These results are supported by previous research: acidification of the diet in a study by Ching et al. (1989) also led to lower apparently digested phosphate and reduced phosphate retention [24]. Similarly, Pastoor et al. (1994) observed lower urinary phosphate concentrations when adding $CaCl_2$ compared to $CaCO_3$ to the diet of healthy cats [44].

A possible explanation is the existence of chemical interactions between different mineral compounds in the feed. In the present study, NaH_2PO_4 was used in combination with either $CaCO_3$ alone or with a mixture of $CaCO_3$ and $CaCl_2$, respectively. In aqueous solutions, these compounds can react as follows:

nCAB: $NaH_2PO_4 + CaCl_2 \rightarrow Ca(H_2PO_4) + NaCl$

pCAB: $NaH_2PO_4 + CaCO_3 \rightarrow Ca_3(PO_4) + H_2O + 3\ CO_2 + 2\ NaOH$

In diet nCAB, the described reaction results in calcium hydrogen phosphate (DCPA, $Ca(H_2PO_4)$) formation, while in diet pCAB it is more likely that tricalcium phosphate (TCP, $Ca_3(PO_4)$) is formed. In acidic solutions, such as those found in the cat's stomach, TCP exhibits a considerably higher solubility than DCPA [45, 46]. As solubility and bioavailability of minerals are closely connected, the higher solubility of TCP could explain the increase of apparently digested phosphate in diet pCAB, irrespective of the diet's CAB.

In alignment with the amount of apparently digested phosphate, the postprandial serum phosphate values increased in group pCAB and were significantly higher than in CON, exceeding the reference range in 5/11 cats. Consequently, the postprandial sCaxP rose above the threshold of 55 mg^2/dl^2 introduced by Block et al. (2000) in 9/11 cats [34]. An increased sCaxP should be avoided because it increases the risk of soft tissue calcification [47] and is negatively correlated with life expectancy for example in human and canine kidney patients [48, 49].

Serum FGF23 values, an early marker of CKD, were significantly increased pre- and postprandially in group pCAB, but not in group nCAB despite the same supply with inorganic phosphate (NaH_2PO_4+$2H_2O$), causing values above the threshold of 300 pg/ml in 3/11 cats. Presumably, this was caused by the higher amount of apparently digested phosphate and the increased serum phosphate concentration in this group. As a phosphatonin, FGF23 increases renal phosphate excretion in response

to elevated serum phosphate concentrations, thereby regulating phosphate homeostasis [50, 51]. Apart from the direct connection between renal health and the amount of phosphate excreted per nephron [15], increased FGF23 serum values were shown to have additional direct adverse health effects, such as the disruption of vitamin D and bone metabolism [52, 53] or cardiovascular dysfunction [54, 55]. Despite the increased serum FGF23 concentrations in pCAB, the only numerically higher urinary P/Crea ratio [56] (table 4) and absolute amount of urinary phosphate excretion per kg BW in group pCAB compared to nCAB indicate that renal phosphate excretion was only slightly affected. The lower urinary creatinine values in the CAB diets can be explained by the overall lower food intake compared to CON.

Digestibility, availability and consequently potential adverse health effects of inorganic phosphates do not solely depend on their amount and source, but also on the supply with other minerals such as sodium [42]. In this study, significant effects of sodium monophosphate as a source of inorganic phosphate on the phosphate homeostasis were determined only in the diet with a relatively high CAB. However, previous research suggests that diets with a negative CAB might affect health for different reasons, regardless of the phosphate consumption. Several studies in cats [24, 25, 29] demonstrated adverse effects such as metabolic acidosis and alterations of the animal's mineral balance after ingestion of acidifying diets. Consequently, reducing the dietary CAB is not a viable solution to attenuate possible adverse health effects of highly soluble inorganic phosphates added to the diet of cats.

Conclusion

In this study, a dietary CAB of 450 mmol/kg dry DM in a diet containing sodium monophosphate led to a significant increase of apparently digested phosphate, phosphate retention, serum phosphate and serum FGF23. Consequently, potential health risks due to the intake of inorganic phosphates can only be evaluated when extensive information about the composition and ingredients of a diet are considered. Concluding from the results of the present study, additional research regarding possible effects of phosphate containing food additives in combination with other dietary factors is required before postulating a safe upper limit, guaranteeing that inorganic phosphate is unconditionally safe for human and animal consumption.

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