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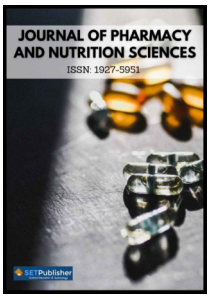

In Vitro Phosphate Binding Capacity of a Dietary Supplement for Dogs and Cats

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Abstract:

Hyperphosphatemia has been recognized as a serious and frequent complication in dogs and cats with Chronic Kidney Disease (CKD). The veterinary approach is mainly based on reducing phosphorus in the diet and with phosphate (P) binders. In this *in vitro* study we compared seven supplements with a different combination of ingredients for dogs and cats at pH 3 and 7 to estimate the P binding capacity overtime. Our results confirmed the best binding capacity of supplements at acid compared to basic condition. The P binding capacity of two products containing mainly calcium carbonate, calcium lactate-gluconate and chitosan was higher compared to the other tested at the same conditions.

INTRODUCTION

Hyperphosphatemia has been recognized as a serious and frequent complication in dogs and cats with Chronic Kidney Disease (CKD) [1]. Phosphorus (P) is absorbed in the gastrointestinal tract (GI) and its level in blood remains constant in healthy animals if there are no changes in their diet. On the contrary, animals with CKD are often subjected to P retention causing hyperphosphatemia and then secondary hyperparathyroidism. The progression of CKD can be reduced by controlling the rise of P blood levels. The veterinary approach consists in changing dietary habits suggesting the use of diets with reduced P content and the administration of P binders [2-5].

There are three main types of phosphate binders available on the market: calcium-containing binders, aluminum-containing binders, and non-calcium-based binders (Lanthanum, Sevelamer) [6].

Calcium carbonate is the most common form of P binder used both in humans and pets because of its usage, efficacy, and lower costs [3-5, 7-9]. It is typically given to patients with CKD. Monitoring the calcium levels to reduce the risk of hypercalcemia [1] and ectopic calcification [9] is advisable in these cases. For these reasons, calcium carbonate has been used in combination with other P binders [9]. Calcium-based P binders are susceptible to pH [9] and are more effective if administered during meals [6].

Aluminum hydroxide belongs to the first era of P binders but data in the '80s showed that a long-term use of aluminum-based binders can lead to intoxication [10]. Non-metallic phosphorus binding resins and other novel agents are the last generation of P binders. Unfortunately, they result to be costly and animals' trials are scarce [6].

Adverse effects of administration of phosphate binders are usually associated with the use of high-dose; therefore, combination of multiple ingredients in a supplement contributes to reducing adverse effects and higher costs. In human studies the use of therapies with calcium-containing P binders showed beneficial effects on patients clinical practice [9].

The aim of our *in vitro* study is the comparison of phosphate binding activity at pH 3 and 7 and at different time points of seven P binder supplements available on the market for dogs and cats.

MATERIAL & METHODS

The seven products (product A-G) used to test binding capacity in this *in vitro* trial are listed in Table **1**.

Table 1: List of Binders (Product A-G) Included in the *In Vitro* Study

Product	Aspect	Composition	Dose / kg body weight/day
Product A	Powder	Calcium carbonate 26%, Calcium lactate gluconate 16%, Chitosan 8%, Sodium bicarbonate 5%, Sodium pyrophosphate, Yeasts [brewer's yeast], Lupin protein meal, Sunflower oil, Vitamins, Maltodextrin.	dog and cat: 0.2 g
Product B	Powder	Calcium carbonate 26%, Calcium lactate gluconate 16%, Chitosan, Sodium bicarbonate 6%, Sodium pyrophosphate, Yeasts [Brewers' yeast], Lupin protein meal, Sunflower oil, Fructo-oligosaccharides, *Lactobacillus acidophilus, Olea europaea* L, Vitamins, Maltodextrin.	dog and cat: 0.2 g
Product C	Powder	Calcium carbonate (9,7%), Chitosan, Lactose, Hydrolyzed soy.	dog and cat: 0.4 g
Product D	Soft capsule	Fish oil (70% EPA-10%DHA) 25%, Gelatine (bovine) 20,49%, Glycerol, Potassium citrate, Krill oil, Sorbitol, Plant and fat oil (Helianthus annuus L.), Mono-e diglycerid of fat acid (plant origin) esterified with organic acids (stearic acid).	dog: 0.14 g cat: 0.28 g
Product E	Oral suspension	Calcium carbonate (3,2%), Chitosan (3,72%), Fish oil, Palmitoylethanolamide (3,72%).	dog and cat: 0.2 ml
Product F	Tablets	Calcium carbonate, Chitosan, Fructo-oligosaccharides, Hydrolysate of chicken liver, Titrated herring oil in EPA e DHA, , Sorbitol, , Polyporus powder (Grifola umbellata (Pers.) Pilat, fruiting body), Magnesium stearate, Maltodextrin.	dog and cat: 0.1 g
Product G	Oral suspension	Calcium carbonate (3,57 %), Magnesium carbonate (0,95 %), Chitosan (0,95 %), Fish hydrolyzed proteins (1,9 %), Astragalus (9,5 %), Meat and byproducts (poultry), Glyceric ester for fat acids.	dog and cat: 0.5 ml

Filter papers were supplied by Whatman plc (Little Chalfont, Buckinghamshire, UK) and 0.2 µm filters were supplied by Merck (Burlington, MA, US). All other materials and chemicals used in this experiment were purchased from Sigma-Aldrich (Burlington, MA, US).

The laboratory method chosen for performing this study followed the one published by Sheikh and colleagues (1989, [11]). To obtain each of the samples analyzed, starting from the seven binders chosen for the study, we followed the quantity of product per kg of body weight (BW) per day indicated on the label. The quantity of product tested is therefore independent of the ingredient concentration with the function of P binder, but is linked to the dose. This choice allows for comparison among the P binding capacity of the seven products on the market at the same quantity/kg BW/day. A sample based on 1.43 g of NaH2PO4- H2O (0.32 g of elemental P) dissolved in 570 mL of deionized water was used as control. A total of 1.43 g of NaH_2PO4- H_2O (0.32 g of elemental P) was dissolved in 570 mL of deionized water. Each binder was dissolved or suspended in deionized water to a volume of 30 mL. The binder solution or suspension was added to the P solution to give a final volume of 600 mL. For each binding essay, two P solutions containing the binder were titrated, by adding HCl or NaOH, up to a pH value of 3 and 7. Samples were poured into sterile beakers, covered with plastic wrap, incubated in a water bath at 37°C, shaking at 20 cycles per minute. As there was a drift in pH over time, the pH of the solutions was checked at regular time intervals and samples were titrated to their initial pH (3 or 7) by adding HCl or NaOH.

Aliquots were taken at regular time intervals from the beakers and were centrifuged at 4°C, at 3,000 rpm, for 30 min. The supernatant obtained was filtered through filter paper, followed by a filtration through a 0.2µm filter. P concentration was assayed by the method of King (2014, [12]). For each binder, reactions were stopped when no more than a 5% increase in binding was observed over a period of 7 days of incubation. Essays for each binder and control were performed in triplicate.

Data analysis were performed using Microsoft Excel (2020).

RESULTS & DISCUSSION

The novelty of this study is the comparison of the *in vitro* binding capacity at two pH of seven products available on the market, characterized by different association of ingredients at different concentration. Human *in vitro* studies had examined the P binding capacity of combination of calcium carbonate with other P binders. Interestingly, most of them did not show the expected increase in P binding capacity [9]. The pH conditions are very relevant when testing an orally administered supplement. In this study each of the tested products showed different binding properties based on the pH condition and experimental time. In Table **2** we report the binding capacity expressed in µmol (mean of the 3 samples tested) and percentage of the amount of bounded P for each product at each time point (T5 to T360) at the two pH (pH 3 and pH 7) (Figure **1**). Data showed all products working better at pH 3 and mainly reaching the maximum binding capacity after 240-300 min except for product D (over 350 min). This could indicate a negative effect of high pH on the binding proprieties of the products. Indeed, the administration of a P binder straight after a meal is strongly recommended as it helps decreasing the pH of the stomach and then improving the supplement activity. If the patient is fed more than once daily, the total daily dose of the phosphate binder should be divided in portions administered with every meal. Administering the binders away from meal time markedly reduces their effectiveness [13]. In the literature, products containing calcium carbonate are known to work better in an acid environment [9] while aluminum based binders work better in a basic [11].

Product A and B showed the highest binding capacity for the same quantity/kg of BW when compared to the other products (Figure **1**). The binding capacity for all the products observed at the end of the study (T360) is still remarkable but, as expected, a decrease has been observed in most cases due to the buffer capacity of binders [13, 14]. P absorption happens in the small intestine 3-9 hr after the stomach is empty. So having a binder able to bind high percentages of P before that time is an advantage for CKD patients. The clinical goal of giving this type of supplementation (mainly containing calcium based-binders) is to bind the P contained in the diet, thus effectively lowering the absorbable P content in the ingested food [13]. It is remarkable the binding capacity at pH 3 reached by product A and B (around 60%) after only 1 hr, compared to the other products. Furthermore, these two products work faster and more efficiently under the same conditions and based on the same dose/kg/day. This probably means that there was a synergic effect of the different ingredients with P binding capacity

Table 2: Phosphate Concentrations in µmol at each Time Point (t0 to t360) over 5 hr Calculated for each Binder (Product A-G). Percentage (perc) Phosphate Binding at each Time Point (t0 to t360) over 5 hr Calculated for each Binder (Product A-G)

Product	pH	µmol_t0	µmol_t5	µmol_t20	µmol_t60	µmol_t120	µmol_t180	µmol_t240	µmol_t300	µmol_t360
Product A	3	0.00	235.47	261.21	308.22	353.57	368.62	370.81	385.60	380.48
	7	0.00	113.19	124.05	146.60	168.00	204.11	231.36	269.98	274.49
Product B	3	0.00	232.02	264.29	298.66	351.23	362.55	376.89	386.03	382.82
	7	0.00	108.10	128.56	149.13	177.25	202.62	237.08	268.20	281.51
Product C	3	0.00	125.10	216.40	230.41	241.43	241.25	251.55	265.08	259.10
	7	0.00	92.51	119.06	155.34	186.19	198.90	215.33	217.46	227.60
Product D	3	0.00	0.00	34.54	61.19	85.26	122.88	151.42	170.51	180.34
	7	0.00	0.00	1.91	25.28	42.19	56.30	82.81	84.26	97.69
Product E	3	0.00	116.17	218.76	225.05	224.10	236.82	253.46	253.12	243.76
	7	0.00	101.99	122.38	150.67	182.90	203.44	216.45	213.55	218.84
Product F	3	0.00	116.48	220.84	221.04	229.46	238.86	248.51	257.89	254.25
	7	0.00	98.31	115.91	153.15	178.10	199.34	201.58	214.29	216.98
Product G	3	0.00	173.98	213.58	254.37	287.18	297.27	322.07	315.02	317.44
	7	0.00	82.33	106.68	127.65	142.92	178.09	202.95	245.87	248.25
Control	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Product	pH	perc_t0	perc_t5	perc_t20	perc_t60	perc_t120	perc_t180	perc_t240	perc_t300	perc_t360
Product A	3	0.00	47.09	52.24	61.64	70.71	73.72	74.16	77.12	76.10
	7	0.00	22.64	24.81	29.32	33.60	40.82	46.27	54.00	54.90
Product B	3	0.00	46.40	52.86	59.73	70.25	72.51	75.38	77.21	76.56
	7	0.00	21.62	25.71	29.83	35.45	40.52	47.42	53.64	56.30
Product C	3	0.00	25.02	43.28	46.08	48.29	48.25	50.31	53.02	51.82
	7	0.00	18.50	23.81	31.07	37.24	39.78	43.07	43.49	45.52
Product D	3	0.00	0.00	6.91	12.24	17.05	24.58	30.28	34.10	36.07
	7	0.00	0.00	0.38	5.06	8.44	11.26	16.56	16.85	19.54
Product E	3	0.00	23.23	43.75	45.01	44.82	47.36	50.69	50.62	48.75
	7	0.00	20.40	24.48	30.13	36.58	40.69	43.29	42.71	43.77
Product F	3	0.00	23.30	44.17	44.21	45.89	47.77	49.70	51.58	50.85
	7	0.00	19.66	23.18	30.63	35.62	39.87	40.32	42.86	43.40
Product G	3	0.00	34.80	42.72	50.87	57.44	59.45	64.41	63.00	63.49
	7	0.00	16.47	21.34	25.53	28.58	35.62	40.59	49.17	49.65
Control	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

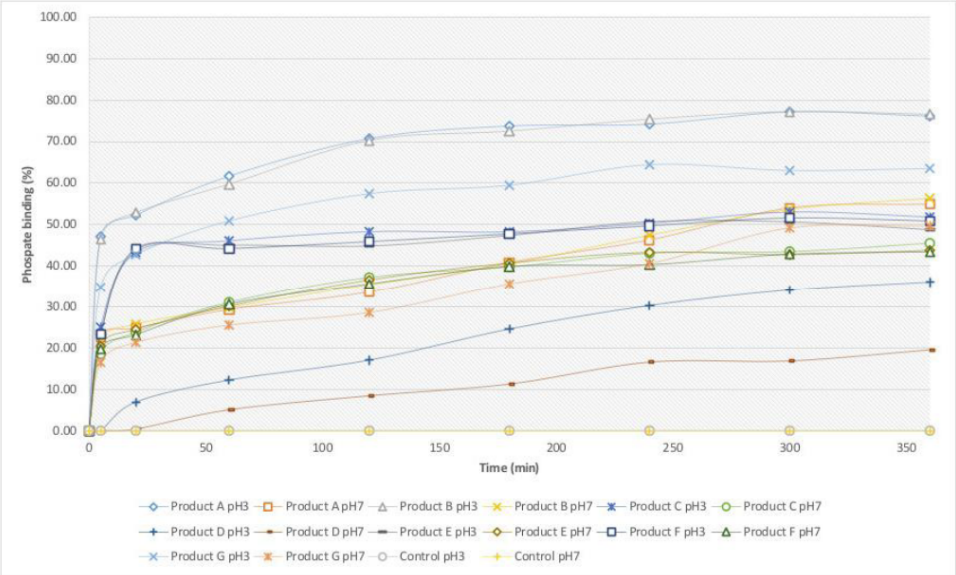

Figure 1: Percentage of the amount of bounded P for each product at each time point (T5 to T360) at pH 3 and 7.

included in the two formulations, specifically calcium carbonate, calcium lactate-gluconate, and chitosan. Interestingly, Products A and B are the only two having these three ingredients combined together.

CONCLUSIONS

This *in vitro* study confirms the best P binding capacity of all the tested supplements for pets at acid compared to basic condition. The P binding capacity of the two products A and B is higher compared to the other ones tested at the same conditions, this being relevant for the clinical practice. It would be interesting to investigate the *in vitro* synergic effect of the different ingredients/additives included in each of the seven products compared to the single binding ingredient calculated at the same concentration as in the product.

CONFLICT OF INTEREST

No conflict of interest.

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