Published by SET Publisher

Journal of Pharmacy and Nutrition Sciences

ISSN (online): 1927-5951

Acid Suppressant Activity of Feed Dietary Supplements for Dogs: An *In vitro* Study

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Article Info:

Keywords:
Supplement,
pets,
antacid,
nutraceutical.

Timeline:
Received: June 07, 2023
Accepted: July 31, 2023
Published: August 30, 2023

Citation: Bruni N, Martello E, Gabriele V, Perondi F. Acid suppressant activity of feed dietary supplements for dogs: An in vitro study. J Pharm Nutr Sci 2023; 13: 38-44.

DOI: https://doi.org/10.29169/1927-5951.2023.13.04

Abstract:

Gastritis is a common finding in pets and acid suppression plays an important role in its management. Acid suppression is the most important component in the treatment of gastritis and prevention of its complications. There are many drugs commonly used in veterinary medicine but the use of natural feed supplements is increasing. The aim of this *in vitro* study is the comparison of four supplements based on a combination of antacid salts and natural products at two pH conditions (pH 2 and 4.5) and at five time points. In addition, we wanted to confirm the formation of the gel during the test. All the products demonstrated acid suppression activity. In particular, product A showed the best performance. All the products except one (product B) formed gel after five minutes from the beginning of the experiment confirming their protective activity. Based on this preliminary results, the product A resulted to be the most promising antacid and potentially gastric protective product compared to others.

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INTRODUCTION

Gastritis is a relative common finding in pets (about 35% in symptomatic dogs) [1,2]. Irritation, infection, or injury (i.e. drugs, chemicals, erosion, ulceration) of the gastric mucosa stimulates the release of inflammatory and vasoactive mediators with subsequent damage of gastric epithelial cells and increase of gastric acid secretion impairing gastric barrier functions [2]. Gastritis is characterized by acute or chronic vomiting secondary to inflammation of the gastric mucosa [1,2]. Acid suppression plays an important role in the management of gastric disease by preventing further complications [3].

The treatment for the gastritis depends on the specific cause (i.e. antiparasitic agents, discontinuation of a drug, removal of an allergen) and the appropriate choice of medication is based on reducing symptoms (eg, increase gastric acidity in uremic gastritis, gastric hypomotility in vomiting) and on understanding the mechanism of action of each drug to avoid negative effects [4]. Acid suppression is the most important component in the treatment of gastritis and prevention of gastric ulcers [5]. There are main drug classes currently in use in veterinary medicine for acid suppression such as Histamine-2 (H2) receptor antagonists (i.e. cimetidine, ranitidine and famotidine), proton pump inhibitors (i.e. omeprazole) [4,5], misoprostol and sucralfate [6]. In addition, inorganic and insoluble salts (such as aluminum hydroxide, calcium carbonate, and magnesium hydroxide) are among the oldest gastrointestinal (GI) protectants reported in the literature with acid suppression activity [6]. Recent studies documented adverse effects of long-term supplementation and discontinued administration of some antacid drugs (such as proton pomp inhibitors) in both human and animals [6,7]. Beside this, antacids are widely used globally in humans for the treatment of symptoms of acidity conditions because of their rapid action and safety [8]. Unfortunately, inappropriate prescription of acid suppressants is a common practice in veterinary medicine. The adverse effects of antacid salts are rarely reported in veterinary medicine and mainly referred to aluminum toxicity in case of long term administrations [6]. Finally, an increasing interest in nutraceuticals for both the management and prevention of gastric ulcere syndrome in veterinary medicine has been documented in recent years [9]. Positive effects have already been observed for some nutraceuticals (i.e. liquorice, *Aloe vera*, mucilages) in the management of different diseases such as gastric diseases [10-11] being a promising addition to medical treatments [9]. Furthermore, some products able to form gel either alone or in combination allow protection of gastric mucosa:natural ingredients (i.e. *Aloe vera*, Guar, Psyllium, Fenugreek), carbohydrate polymers (i.e. pectin), and salts (i.e. sodium alginate) [11-13].

The aim of this *in vitro* study is the comparison of four supplements based on a combination of natural products. We evaluated the reduction of the pH of a solution simulating the production of acids in dog stomach. We recorded the change in pH at five different time points over 60 minutes (T0-T60) in a solution of pH 2 and 4.5. In addition, we observed the formation of the gel at each time point.

MATERIALS AND METHODS

Preparation of the Solutions for the Experiments at Two Set pH (pH 2.0 ± 0.2 and pH 4.5 ± 0.2)

We equiped a 1-litre Becker with a pH measurement probe (Metter LL Solidrode Pt 1000 PP type), clean glassware. The device was an automatic Mettler 014 with temperature compensation and digital reading. A suitable sized stir bar was placed in the beaker and then placed on a speed controlled magnetic stirrer. The pH meter and the magnetic stirrer were calibrated at the time of use with buffer solutions and verification of the number of revolutions. A quantity of 900 ml of water was added to the Becker and placed under strong agitation (400 rmp). Then, we added under a fume hood with strong agitation 37% HCL, using a graduated pipette with a 5 ml discharge. This passage was performed until reaching a value of pH 2.5 ± 0.2 for the first test or a value of pH 4.7 ± 0.2 for the second test. Using a double-notch pipette, always preventing micro additions of the same fuming HCL 37%, the pH was brought to the reference value of pH 2.0 ± 0.2 for the first test or a value of pH 4.5 ± 0.2 for the second test. In case the pH dropped below the target value, we used a 0.5 ml double-notch pipette, adding a ready-to-use 0.5 M NaOH solution (Carlo Erba lot CA1235).

The pH was read and needed to confirm that the target pH has been reached and then stabilized for 10 minutes ± 2. If the pH dropped below the target value, we used the solution described above. On the other hand, if it raised we made an addition, using pipettes with double notches, of 0.5 ml ready-to-use solution of HCl 0.1 M Carlo grass CB3454.

Finally, r the solution was transferred thus stabilized it into a 2-liter flask, identify it with label, and close it securely.

Preparation of the Samples from the Four Commercial Supplements under Study

In case of powder products, the weighing was carried out directly using a Mettler XS205 analytical balance in vials. In the case of tablet products, before weighing on the same scale used for the powder products, the tablet was crushed with the aid of a ceramic mortar and pestle, homogenized avoiding overheating. The weighing was then performed as in the previous case. In both cases, the vials were thoroughly washed with several rinses of the same buffer solution using a graduated pipette with continuous drainage after the transfer of the powder. At least three rinses were performed.

In the case of the paste products, the paste was put directly in a crystallizer. To completely transfer it to the aforementioned glass beaker, the crystallizer was thoroughly washed using the same pH 2.0 ± 0.2 or pH 4.5 ± 0.2 solution with a pipette.

Testing the Acid Suppressant Activity of the Supplements in a pH 2.0 ± 0.2 and a pH 4.5 ± 0.2 Solution

Two series of tests were carried out, one using the solution at pH 2.0 ± 0.2 and one at pH 4.5 ± 0.2. For each measurement, three repetitions were carried out using a new sample weighted each time.

A total of 50 ml of the solution at pH 2.0 ± 0.2 for the first test or pH 4.5 ± 0.2 for the second test were transferred into 15 Class A glass backers and placed a magnetic stir bar inside. The pH of the solutions contained in each beaker was measured before adding the product.

For each test slot the operations below were repeated for three weighs of the product under investigation.

The glass beaker was placed on the magnetic stirrer. The stirring was activated providing at 300 rpm. After weighting an accurately measured amount of each of the selected products corresponding to the indicated daily dose, we transferred the powder into the same beaker and left it stirring for at least 5 minutes and observed the appearance of the solution - suspension (presence of gel, absence of gel, partially formed gel). After evaluating the appearance, we put it back into vigorous agitation and measured the final pH at each time point. To measure the pH, we turned off the stirring, inserted the probe into the suspension/solution and recorded the value read directly in the provided Table (Table **2**). After reading, the stirring was reactivated and after 15 minutes we carried out the physical observation of the solution/suspension recording the pH value. The same activity was repeated at 30 and 60 minutes. The times indicated were considered with an acceptance range of 2 minutes.

RESULTS & DISCUSSION

This study compared *in vitro* suppressed acidity of two different pH solutions (2 and 4.5) simulating dog stomach acidity by four natural products at different time points. The composition of the four products are reported in Table **1**. Table **2** reports pH at different time points (T0, T5, T15, T30, T60) of the products (product A, B, C and D) using a starter solution at pH 2 and 4.5.

Data showed that all products are working better and mainly reaching the maximum suppressed acidity starting at pH 4.5 (Table **2**).

All the products showed a little increment of the final pH at different time points. Interestingly, pH for product A tended to be higher than the others, especially at pH 4.5 reaching a maximum of 5.19 at the end of the study (Table **2**, Figure **1**). All the other products reached a maximum pH at the end point of 4.9 (less than 5) showing a little less acid suppressed activity in this *in vitro* condition.

All the tested products included calcium carbonate at different dosages (Table **1**). Calcium carbonate is recognized as a commonly used antacid in human medicine, one of the most effective antacids known to have a rapid, long-lasting, and effective neutralizing action [8,14]. Calcium carbonate reacts with gastric HCl, then carbonate anions bind to free protons from HCl, with decreasing protons concentration in the stomach and consequently raising the pH [8].

An *in vitro* study, using a validated human stomach model, has demonstrated the antacid fast-acting and strong acid-neutralizing properties of calcium/ magnesium carbonate recording an increase in pH, rapidly achieved (within 40 seconds) [14]. The same study also showed that both calcium carbonate and magnesium carbonate have high anti-peptic activity [14].

Table 1: List of Product (Product A-D) to Reduce pH Included in the *In vitro* Study

Product	mg/kg
Product A	
Calcium carbonate	50,000
Camelia sinensis	30,000
Aloe vera	6,000
Trigonella foenum grecum extract	13,000
Guar seed flour	75,000
Plantago ovata (*Psillio*) extract	19,000
Magnesium hydroxide	38,000
Glycyrrhiza glabra extract	6,000
Methylsulfonylmethane	31,000
L-Treonine	63,000
Other ingredients (*Optimizor Uranus*, Cellulose microcrystalline type 2, brewer's yeast, Colloidal silica E551b,Compritol, Magnesium Stearate)	669,000
Product B	
Sodium alginate	250,000
Sodium bicarbonate	125,000
Calcium carbonate	35,000
Other ingredients	590,000
Product C	
Calcium carbonate	150,000
Sodium bicarbonate	100,000
Althaea officinalis root	100,000
Carob flour	100,000
Carum carvi	75,000
Sodium alginate	35,000
Ananas comosus extract (0,3% Bromelina)	30,000
Glycyrrhiza glabra L. extract (10% Glycyrrhizic acid)	15,000
Zingiber officinalis Rosc extract (1% Gingerolols)	10,000
Other ingredients	385,000
Product D	
Calcium carbonate	80,000
Sodium bicarbonate	70,000
Chondroitin sulphate	40,000
Althea officinalis products	40,000
Other ingredients (milk serum powder, pectin, *Lactobacillus reuteri* inactivated, *Ceratonia siliqua* L., maltodestrin, sodium hyaluronate, sorbitol, Glycerol, animal protein hydrolyzed (poultry origin)	750,000
Additives (Sodium alginate, Microcrystalline cellulose, Colloidal silica, Xanthan gum)	20,000

Table 2: Raw Data of the pH Values (Three Repetitions) Reached by the Four Tested Products (Products A to D) and the Control Sample at each Time Point (T0, T5, T15, T30, T60) at Two Conditions using a Solution at pH 2 and pH 4.5

Product	pH 2					pH 4.5				
	T0	T5	T15	T30	T60	T0	T5	T15	T30	T60
Product A	2.08	2.23	2.41	2.60	2.63	4.51	4.75	4.83	5.09	5.19
	2.02	2.28	2.36	2.59	2.61	4.55	4.81	4.88	5.05	5.06
	2.09	2.32	2.37	2.56	2.64	4.50	4.83	4.86	4.90	5.15
Product B	2.00	2.16	2.19	2.45	2.49	4.58	4.61	4.76	4.81	4.92
	2.04	2.10	2.11	2.41	2.51	4.57	4.64	4.65	4.76	4.90
	2.04	2.08	2.17	2.29	2.41	4.51	4.63	4.65	4.73	4.89
Product C	2.07	2.14	2.27	2.29	2.34	4.56	4.66	4.71	4.85	4.99
	2.00	2.20	2.31	2.35	2.43	4.56	4.62	4.68	4.75	4.87
	2.08	2.08	2.20	2.27	2.34	4.54	4.65	4.77	4.81	4.94
Product D	2.00	2.02	2.11	2.40	2.43	4.52	4.56	4.68	4.76	4.83
	2.04	2.15	2.19	2.34	2.39	4.50	4.66	4.72	4.83	4.89
	2.01	2.08	2.11	2.27	2.41	4.52	4.70	4.70	4.72	4.94
Control	2.04	2.03	2.03	2.05	2.08	4.56	4.53	4.53	4.51	4.55
	2.01	2.08	2.04	2.01	2.00	4.53	4.58	4.57	4.55	4.56
	2.03	2.06	2.02	2.02	2.02	4.52	4.50	4.59	4.54	4.54

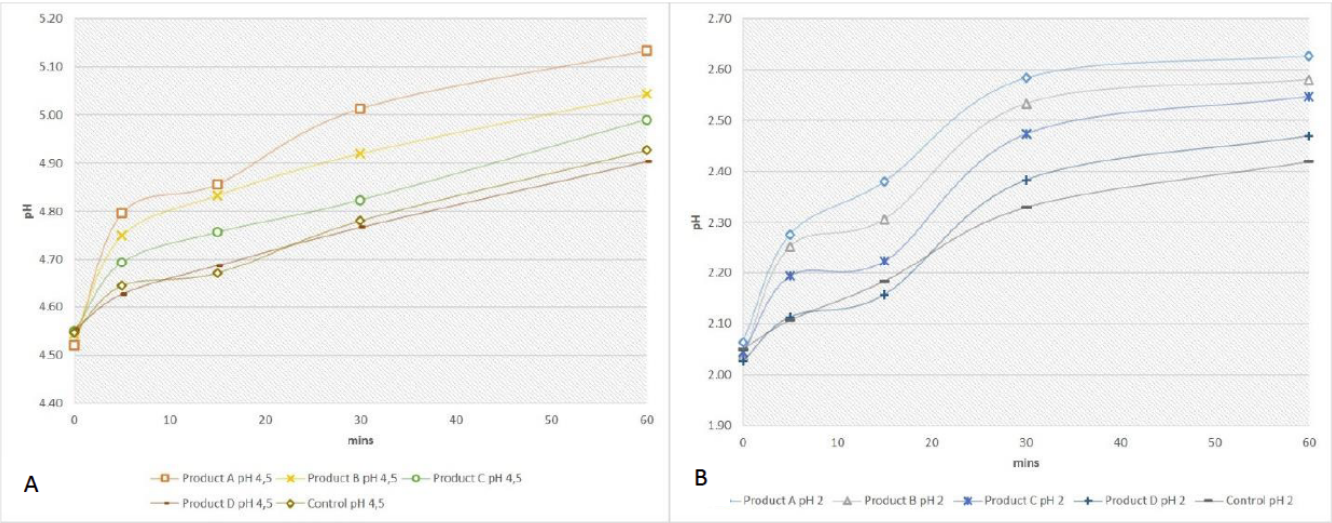

Figure 1: (**A**, **B**): Plot of the mean pH values reached by the four tested products (Products A to D) and the control sample at each time point (T0, T5, T15, T30, T60) at two conditions using a solution at pH 4.5(**A**) and pH 2 (**B**).

Several reports have suggested that the basal (fasting) gastric pH of dogs is higher or more variable than the basal gastric pH of humans [3]. Dogs have lower basal acid secretory rates but considerably higher peak gastric acid responses compared to humans [6]. Beside this, some recent studies reported fasting gastric pH was comparable among dogs, cats, and humans [3, 15,16]. Human studies also reported that gastric pH ≥3 and ≥4 is considered the ideal baseline for encouraging healing of GI disease [15].

As showed in Table **2**, the tendency of reducing acidity of the product A is relatively higher than the other products. It is to be noted that product A is characterized by the combination of carbonate calcium with another antacid salt, magnesium hydroxide, this

combination could have enhanced the alkalizing activity.

Magnesium hydroxide reacts rapidly with gastric HCl to produce magnesium chloride and water with suppressed acid activity [8]. Although Antacids principal mechanism of action is the reduction of gastric acidity, they may also promote mucosal defense mechanisms by stimulating mucosal prostaglandin production and decreasing pepsin activity in the stomach [17,8]. The other products included in this study (Products B, C and D) have sodium bicarbonate among the ingredients at different dosages as the antacid compounds associated to calcium carbonate (Table **2**). The natural ingredients included in the products' formulations were liquorice (products A and B), *Aloe vera* (products A and B), Althea (products B and C) and Threonine (products A) which also have antioxidant proprieties and could have a synergic effect improving the overall acid suppressant activity.

For example, several studies in human medicine proved the efficacy of liquorice as a gastro-protective activity [18], because liquorice extract seems to promote the suppression of acid secretion, the increase of mucine secretion and the release of PGE2 [11]. The threonine is also involved in the production of protective mucus at the gastro-enteric level and the *Aloe vera* has recently been demonstrated to be anti-inflammatory, cytoprotective and mucus-stimulat [11]. In particular, anti-inflammatory effects of *Aloe vera* were reported in horses, showing an increase in the perfusion of gastric mucosa, a reduction of vasoconstriction and a promotion of angiogenesis and facilitation of the healing of ulcers [19,11].

Aloe vera, liquorice and threonine characterized by the above mentioned proprieties, could be the responsible of the improvement in the suppressant acidity together with the other antacid ingredients present in the tested products (calcium carbonate, magnesium hydroxide and sodium bicarbonate).

A recently published clinical trial in horses affected by Equine Gastric Ulcer Syndrome (EGUS), reports the efficacy in reducing the number and severity of ulcerative lesions after administration of a feed supplement containing similar components (calcium carbonate, Magnesium hydroxide, *Aloe vera*, liquorice and threonine) [11].

In this *in vitro* study we reported the potential capability of these natural feed supplements to have a protective effect on the gastric mucosa. In particular, components responsible of gel formation included in these tested products (Aloe vera, Guar, Psyllium, fenugreek, pectin and sodium alginate) formed a viscous gel when in an acid environment. This protects the mucosa from the effects of the acidity [11,13]. According to recent *in vitro* studies that simulate digestion, it has been demonstrated that the mucus retains its structural integrity due to its ability to resist acidic conditions and enzymatic breakdown throughout the digestion process [10]. In addition, other studies have shown that the apparent viscosity in the stomach increases as a function of the concentration of mucilage and given that the mucilage is a functional ingredient that can help delay gastric emptying and helping digestion [10].

As reported in Figure **2**, all the products except product B showed gel formation during the study time starting from T5. In particular, product A contains numerous natural components with gel forming proprieties (*Aloe vera*, Guar, psyllium and fenugreek) and this has already been reported in a previous *in vivo* study in horses [11].

The first limitation of this study is the evaluation of the tested products only *in vitro*. This can only help describing the suppressant acid activity with gel

Figure 2: Visual evaluation of the gel formation in the four products (Products A to D) and the control sample at T30 using a solution at pH 4.5.

formation of these products and not the potential antinflammatory, antioxidant and cytoprotective activities. On the other hand, at our knowledge, there are no other reported *in vitro* studies in veterinary medicine comparing the acid suppressant activity of feed dietary supplements for dogs.

CONCLUSION

Based on this preliminary results, the product A resulted to be the most promising antacid and potentially gastric protectant product compared to the others. In addition, it could be interesting to perform *in vivo* studies to evaluate the efficacy of the four products as anti-acid, anti-inflammatory and mucosal protective in dogs affected by gastric diseases.

CONFLICT OF INTEREST

No conflict of interest.

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