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Manuscript

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Trace element measurement for assessment of dog food safety

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ABSTRACT

The quality of dog diets depends on adequate ingredients capable of providing optimal nutrition and free of contaminants, for promoting long-term health. Trace elements in ninety-five samples of dry food for dog puppies (n=32) and adults (n=63) of various brands were measured using instrumental neutron activation analysis (INAA). The mass fractions of most elements were within the permissible limits for dogs. Aluminum, antimony and uranium presented fairly high levels in some samples, which may imply health risks. Aluminum mass fractions ranged from < 21 mg/kg to 11900 mg/kg, in same brand, super premium dog food. Antimony mass fractions ranged up to 5.14 mg/kg, with the highest values measured in six samples of dog food from the same producer. The mass fractions of uranium was found up to 4 mg/kg in commercial brands from five different producers.

Keywords: aluminum, antimony, uranium, food safety, zeolites, phosphate rocks

INTRODUCTION

Cancer, allergies, neurological diseases, skin disorders, pulmonary, renal and liver failures are common in pets like in man. Pet longevity, owner's habits, nutritionally unbalanced pet food and candy could all contribute to the increased occurrence of such illnesses. Also the limited size of the gene pool of some pedigree pets has its impact on the incidence of certain diseases (Dog Breed Health 2016). Pets need a complete and balanced diet, which is somewhat different at each stage of life (Hodgson 2016). As such, it is extremely important to choose a product capable of providing optimal nutrition, with all necessary substances at adequate levels (Anfalpet 2014) and free of contaminants, for promoting long-term health.

The safety of pet diets has received much attention (Romanini 2010), since a major recall of their food in the United States due to melamine contamination resulting death of animals. In recent years, the pet food market has expanded and a wide variety of new products has been introduced, with different ingredients, processing and preservation methods. The use of low quality raw ingredients can introduce residues of pesticides, mycotoxins, and hazardous chemical elements in the pet food chain. Such undesired components can result in both toxic effects and nutritional deficiencies with an impact on the animals' skeleton, nerve system, metabolism, occurrence of allergies, behavioral and other health problems (Martisen and Casper 2013).

The main components of pet food are carbohydrates, fatty acids, proteins, vitamins, fibers, water and minerals (i.e., macro and trace elements). In this context, the measurement of macro and trace elements is important when evaluating the quality of pet food. As an example, a dog needs a high daily intake of calcium and phosphorus, at a mass ratio of 1.2:1, while in plain meat this ratio is in the order of 1:17. Too high or low chemical elements in food can disturb the pet metabolism and can even cause acute toxicity at extreme levels. The research described in this paper deals with such an orientation on the trace element levels in dry dog food commercially available in Brazil. Instrumental neutron activation analysis (INAA) was used allowing a wide evaluation due to its multi-element capacity (Greenberg et al. 2011).

EXPERIMENTAL

Ninety-five samples of dry dog food for puppies (n=32) and adults (n=63) of thirty-six brands were acquired in the local market of Piracicaba city, São Paulo, Brazil. After homogenization, one 350 g subsample of each dog food package of 1 kg was reduced to particle size lower than 0.5 mm in a titanium knife mill, Retsch Grindomix GM 200. The powdered material was then homogenized and stored in bottles of high-density polyethylene for analysis by INAA. The moisture content in each material was measured by drying a separate portion of 1 g in an oven at 105ºC, following guidance of the Association of Official Analytical Chemists (AOAC, 2006).

One test portion of 300 mg of each material, from the total 95 samples, was transferred into a high purity polyethylene vial (Posthumus Products, Beverwijk, The Netherlands) for irradiation with neutrons. Ten-milligram pieces of a well characterized Ni-Cr alloy were sandwiched among vials for neutron flux monitoring. Reference materials IAEA V10 Hay Powder and SRM 1577c Bovine Liver were included for quality control. All materials were irradiated for 4 hours at a thermal neutron flux of $7x10^{12}$ cm^{-2} s^{-1} in the nuclear research reactor IEA-R1 of the Nuclear and Energy Research Institute (IPEN), National Nuclear Energy Commission (CNEN), São Paulo. After 4-day cooling time, the induced radioactivity was measured by high-resolution gamma-ray spectrometry at the Nuclear Energy Center for Agriculture, Piracicaba. Mass fractions of the elements and their expanded uncertainties (k=2) were obtained by the k_0 method of calibration, using the Quantu software (Bacchi and Fernandes 2003). The quality assessment aspects of such analytical procedure were addressed elsewhere (Bacchi et al. 2003). Additional samples of 300 mg were individually irradiated during 30 s at a thermal neutron flux of 1.5 $x10^{13}$ cm^{-2} s^{-1} in the nuclear research reactor (Hoger Onderwijs Reactor) of the Reactor Institute Delft, Delft, The Netherlands, using the fast pneumatic transfer system. The induced radioactivity of short half-life radionuclides (half-lives shorter than a few hours) was measured by high-resolution gamma-ray spectrometry after 1 min decay, which allowed for the measurement of additional elements. A neutron flux monitor, obtained by pipetting a zinc standard solution in a filter paper was added to each sample, and counted separately. Mass fraction results and their expanded uncertainties (k=2) were obtained by k_{Zn} method, using the Apollo software, with a validated procedure (Bode and Blaauw 2012).

RESULTS AND DISCUSSION

INAA allowed investigating twenty essential and non-essential elements in the samples of dry dog food. Most elements investigated could be measured in the 95 samples, however in a few cases they are below detection limits. The minimum and maximum values obtained are shown in Table 1, where one can note wide ranges of the mass fractions of aluminum, antimony and titanium whereas for most other elements the minimum and maximum values differ by less than a factor of 10. Arsenic, cadmium and mercury, well-known for their toxicity in animals, were not found at levels that might cause concern, considering the maximum levels established for humans (Codex 2011). Nevertheless, aluminum, antimony and uranium were observed at fairly high levels in some samples, which could be considered of potential risk to dog health. This is discussed below in more detail.

Aluminum

The aluminum results ranged from 48 mg/kg to 11900 mg/kg in adult dog food and from 49 mg/kg to 8500 mg/kg in puppy dog foods. In only one adult food, the aluminum content was below the detection limit, which was 21 mg/kg. In a previous study with dog food conducted in northeastern Brazil (Costa et al. 2013), aluminum levels measured by ICP OES varied between 54 mg/kg and 2835 mg/kg. The highest value was about three times lower than the highest value obtained in this study.

From the total of 95 samples, 24% had aluminum mass fractions higher than 1000 mg/kg (Figure 1). It should be noted that the highest values were observed for dog food of economic and super premium varieties of the same producer. It was reported before that some additives used in animal feed have aluminum in their composition (Sindirações 2012), e.g., zeolites (hydrated aluminosilicates) have been used. Zeolites can promote the adsorption of gas and excess water from the gastrointestinal tract and is therefore applied by the pet food industry for reducing odor and humidity of feces (Lowndes 2014).

Aluminum is extremely reactive with carbon and oxygen, having immense implications for the health of humans and animals (Shaw and Tomljenovic 2013). Despite of its low bioavailability in the body, it is not yet fully understood how aluminum and its compounds interact in the absorption mechanism of the gastrointestinal tract (Berthon, 2002; Codex 2011). However, it is known that adverse effects may occur due to long-term exposure, being the nervous system the most sensitive tissue (Yokel 2000; Exley 2014). Aluminum is the most common toxic element found at high levels in dogs and its main source is pet food (Martisen and Casper 2013). Although maximum limits for aluminum in dog food have not been found in literature or directives, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established the daily intake limit for humans as 1 mg per kg body weight for all aluminum compounds in food, including additives (Codex 2011).

Antimony

The amount of antimony was below the detection limit in about 44% of the samples of adult and 53% of the samples of puppy dog foods. The detection limits ranged from <0.01 mg/kg to <0.04 mg/kg for adult dog food and from <0.01 mg/kg to <0.03 mg/kg for puppy dog food. In the remaining samples, the mass fractions varied between 0.01 mg/kg and 5.04 mg/kg in adult dog food and between 0.01 mg/kg and

5.14 mg/kg in puppy dog food. Atkins et al. (2011) found antimony mass fractions from 0.5 mg/kg to 1.0 mg/kg in dry dog food.

Six samples, i.e. four for adults and two for puppies, of different brands but from the same producer had higher antimony contents (Figure 2). All such samples consisted of multicolored grains, with red, green and yellow pigments. The brand with the highest antimony content was reanalyzed one year later, following the collection of new samples from the market. The antimony values were reduced from above 5 mg/kg for both adult and puppy dog food (first sampling) to below the detection limit of 0.01 mg/kg in the second sampling.

A correlation between elements can be useful to identify their origin. Both chromium and titanium are indicators of possible contamination by metals during the production process. However, it can be seen from Figure 3 that there is no significant correlation between antimony and chromium ($R^2 = 0.0888$) or antimony and titanium ($R^2 = 0.4684$). Therefore, antimony is most likely not from any contamination caused by the equipment in the industrial processing or in the sample preparation, but probably present in a component of the dog food. In principle, antimony migration from packaging is a possibility, as investigated by Smichowski (2008). In Brazil, a legislation was established by ANVISA (2010) with specific migration limits for antimony of 0.04 mg/kg, which should be applied to packages containing dyes and pigments in their formulation intended to be in contact with food.

Antimony is potentially toxic for animals even at low mass fraction (Thompson 2008). Most of absorbed antimony is excreted via urine and feces, but a small part of it may have a long biological half-life. High concentrations of the element can be found in the lungs, thyroid, liver and kidneys. Reproductive disorders, chromosome damage and oncogenesis may be associated with chronic exposure (Tylenda and Fowler 2007).

There is no maximum limit of antimony established for dog food. However, according to the World Health Organization (WHO 2003), the tolerable daily intake of antimony for humans is 6 μg per kg of body weight. Compared to the maximum tolerable value, the antimony intake from the dog food may reach high values. For example, assuming that a dog of 10 kg consumes 350 g of dry food per day, the intake of antimony would be as high as 190 μg per kg of body weight. Considering that antimony is potentially toxic at low mass fraction, can be accumulated in the body and has no known biological function (Peterson et al. 2007; Tylenda and Fowler 2007), it can be concluded that such levels in dog food should not be acceptable.

Uranium

From the total of 95 samples of dog food analyzed, eighteen samples of adult dogs and eight samples of puppies had uranium contents above the detection limit (<0.13 mg/kg). The values in the eighteen samples of adult dog foods ranged from 0.46 to 2.25 mg/kg, while in the eight samples of puppy dog foods varied from 0.34 mg/kg to 3.99 mg/kg. Literature data showed uranium values in pet food (n = 58) ranging from 0.78 mg/kg to 0.86 mg/kg (Atkins et al. 2011). In another study of dry dog food (Elias et al., 2012) uranium was found at levels as high as 4 mg/kg in some of the 34 samples analyzed.

Six samples for adult dogs and five samples for puppies had uranium values ≥1.0 mg/kg (Figure 4). From adult dog foods, two brands are therapeutic and prescribed to dogs with intestinal problems (containing 1.01 mg/kg of uranium) and hypersensitivity (containing 2.25 mg/kg of uranium). In addition, a puppy dog food sample from the super-premium segment contained 3.01 mg/kg of uranium. Such results evidenced that the high uranium content is not related to the low price of some dog foods. From Figure 4, it can be seen that the dog foods with uranium content higher than 1.0 mg/kg originate from five different producers. Two brands with the highest uranium content were again procured at a different opportunity, for evaluating samples from other production lots. For one brand, the uranium mass fraction decreased from 3.15 mg/kg to 0.82 mg/kg, while for the other the mass fraction was similar, with 3.99 mg/kg and 3.53 mg/kg, respectively in the first and second batches collected.

Products derived from phosphate rocks, such as dicalcium phosphate (DCP), are commonly used as additives for animal feed supplementation. Their purpose is to maintain the amounts of calcium and phosphorus to the desired mass fraction ratio (e.g., 1.2:1 for adult dog food). However, material originating from phosphate rock may also contain relatively high amounts of uranium (Casacuberta et al. 2009). In DCPs commercialized at Catalonia, Spain, and used for animal feed, uranium ranged from 80 mg/kg to 250 mg/kg (n = 12), while TCP (tricalcium phosphate), intended for human consumption, showed values from 0.2 mg/kg to 2.0 mg/kg (n = 4) (Casacuberta et al. 2009).

Experiments with dogs, rabbits and rats showed that the animals are sensitive to inhalation and ingestion of uranium compounds with accumulation mainly in the bones, liver and kidney (Keith et al. 2013). Limits for uranium in animal food were not found in literature, however its nephrotoxic effects have been known for a long time, eventually leading to chronic renal failure (Batista 2009; Vicente et al. 2010).

According to the US Environmental Protection Agency (EPA 2010), the daily consumption of uranium in food varies between 0.07 and 1.1 μg for humans. Considering a Labrador dog with 35 kg body mass, for example, which consumes about 500 g of dry dog food per day, the intake could reach 2 mg of uranium, a very high daily intake compared to the safe range published by EPA.

CONCLUSIONS

INAA was useful for the analysis of different types and brands of dry dog foods, allowing the investigation of trace elements that can cause health hazard. The mass fractions for most of the twenty elements determined, including arsenic, cadmium and mercury, were within the permissible limit dog health. However, high mass fractions of aluminum, antimony and uranium were observed in some samples of adult and puppy dog foods. The high contents of aluminum and antimony were associated to one or two brands, while the high uranium contents were observed in brands from five diverse producers. The large difference between minimum and maximum values observed for these elements indicates that the occurrence of high mass fractions can be avoided by a careful selection of ingredients, combined with good production practices and quality control actions. Further investigation is necessary to better understand the presence of the potentially toxic elements found in this study and their consequences to dog health.

The authors are thankful to The State of São Paulo Research Foundation – FAPESP for financial support (Process 10/52425-7).

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Table 1 – Minimum and maximum values (mg/kg) of chemical elements measured in adult dog foods (n=63) and puppy dog foods (n=32). Results expressed in dry mass

	Adult		*Puppy*			*Adult*		*Puppy*	
	Min	*Max*	*Min*	*Max*		*Min*	*Max*	*Min*	*Max*
Al	< 21	11900	49	8500	I	0.9	14	0.7	14
As	< 0.13	0.43	< 0.14	0.56	La	< 0.02	3.32	< 0.02	3.14
Br	2.05	16.90	3.10	15.42	Mn	6.0	111	16	149
Cd	< 1.1	< 2.4	< 1.1	< 2.4	Rb	5.67	25.04	6.50	22.96
Co	0.08	0.82	0.08	0.75	Sb	< 0.01	5.04	< 0.01	5.14
Cr	< 0.20	4.33	< 0.20	8.27	Sc	0.01	1.41	0.01	0.78
Cs	0.02	0.17	0.02	0.19	Ti	< 9.0	2300	< 9.0	2140
Cu	9.0	60	15	64	U	< 0.13	2.25	< 0.13	3.99
Fe	177	862	198	675	V	<0.17	4.88	<0.12	3.24
Hg	< 0.05	< 0.10	< 0.05	< 0.10	Zn	44	554	46	614

Figure 1. Aluminum in samples of dog food that showed results ≥ 1000 mg/kg, expressed in dry mass. The error bars represent the expanded measurement uncertainty (k=2). Bars with similar pattern indicate the same commercial brand

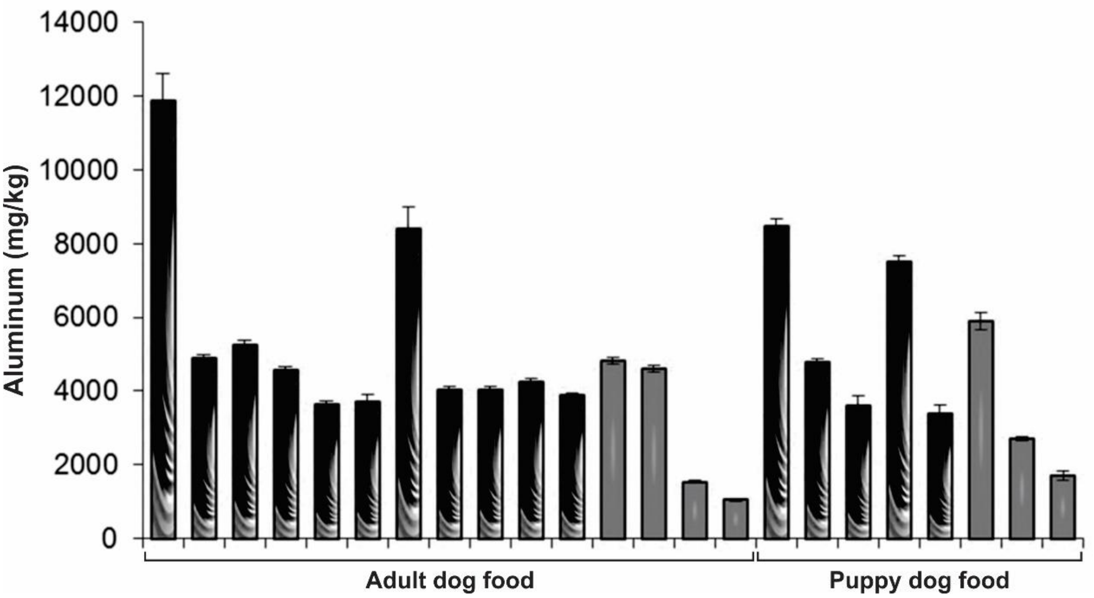


Figure 2. Antimony in samples of dog food found above the detection limit, expressed in dry mass. The error bars represent the expanded measurement uncertainty (k=2). The six highest values were obtained in samples from the same producer

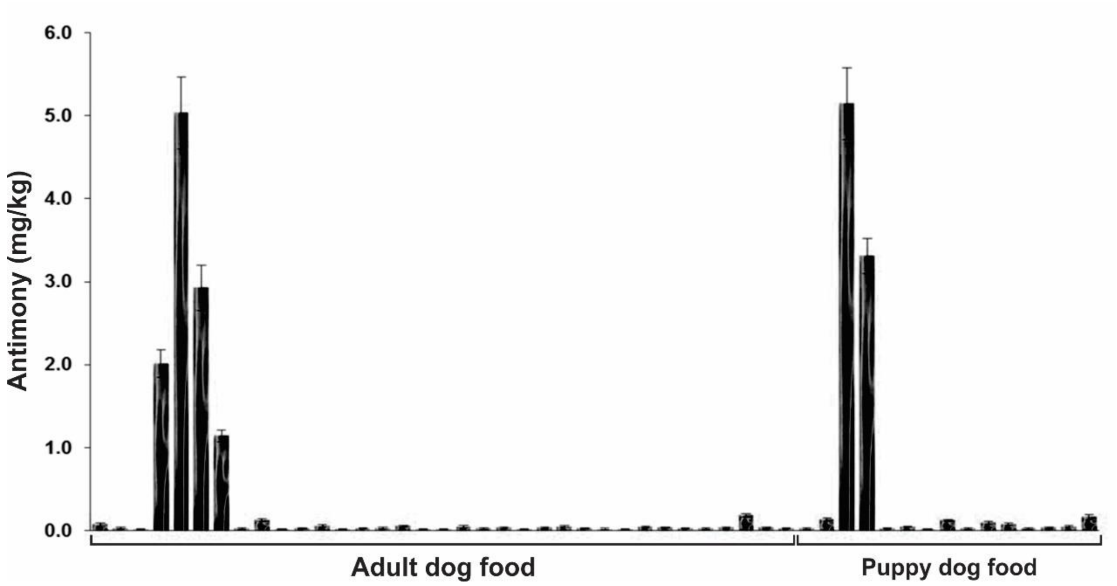


Figure 3. Correlation antimony-chromium (A) and antimony-titanium (B) for the six samples of dog food showing the highest mass fractions of antimony

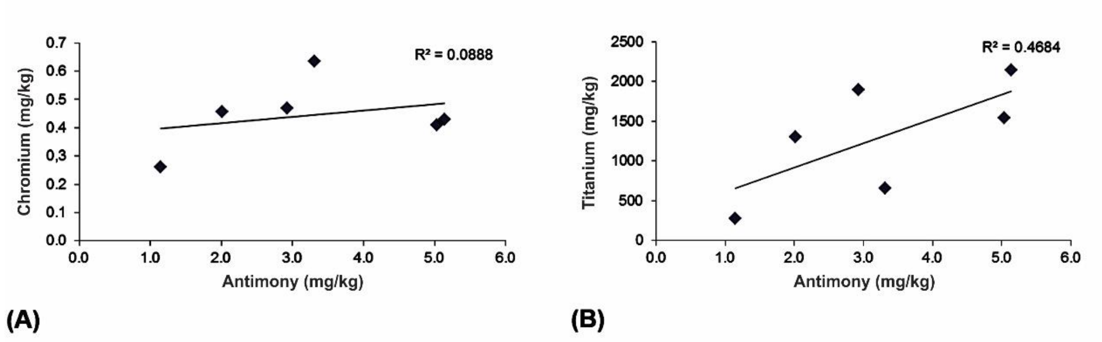
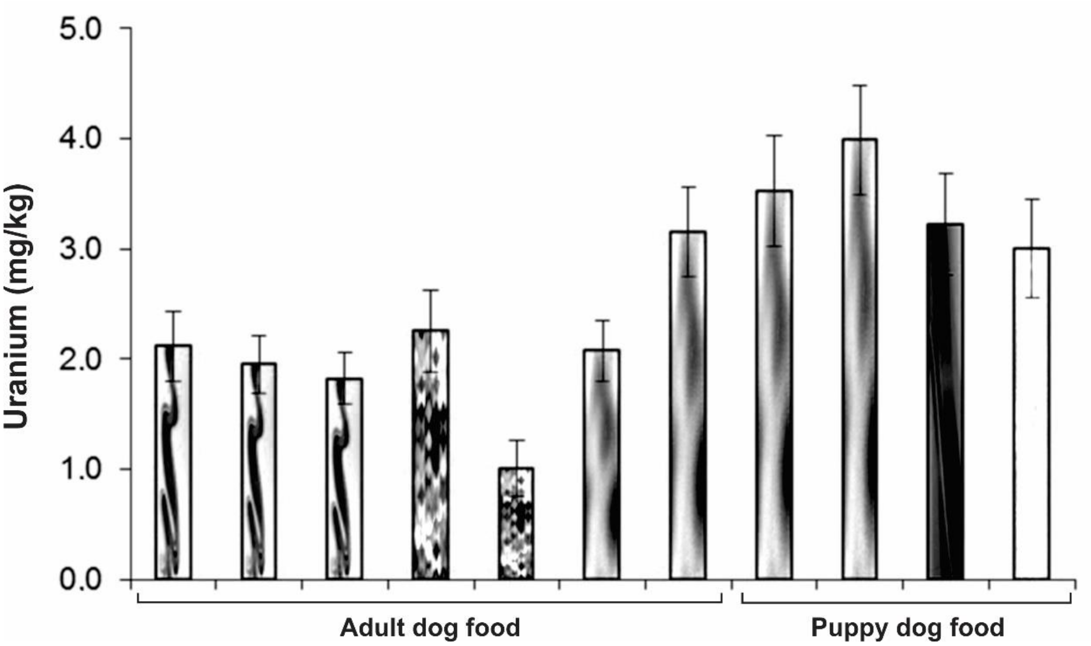


Figure 4. Uranium in samples of dog food showing values ≥ 1.0 mg/kg, expressed in dry mass. The error bars represent the expanded uncertainties (k=2). Bars with similar pattern indicate the same producer



Most of the modifications proposed by reviewers were implemented in the revised manuscript. Here the modifications are detailed, showing text before and after revision, and the questions are answered.

Title

Before: *Multi-element analysis for assessment of dog food safety*

After: *Trace element measurement for assessment of dog food safety*

Abstract

Before: *Trace elements in ninety-five samples of dry dog food for puppies...*

After: *Trace elements in ninety-five samples of dry food for dog puppies...*

Before: *Aluminum mass fractions ranged from < 21 mg/kg to 11900 mg/kg, with the highest values measured in products from the same brand, including super premium dog food.*

After: *Aluminum mass fractions ranged from < 21 mg/kg to 11900 mg/kg, in same brand, super premium dog food.*

Before: *The mass fractions of uranium was found to range up to 4 mg/kg...*

After: *The mass fractions of uranium was found up to 4 mg/kg...*

Introduction

1st paragraph

Before: *Pet longevity, owner's habits, nutritionally unbalanced pet food and pet candy could all contribute...*

After: *Pet longevity, owner's habits, nutritionally unbalanced pet food and candy could all contribute...*

2nd paragraph

Before: *The safety of pet diets has received much attention (Romanini 2010), since a major recall of pet food...*

After: *The safety of pet diets has received much attention (Romanini 2010), since a major recall of their food...*

3rd paragraph

Before: *As an example, a dog needs a high daily intake of calcium and phosphorus, but at a mass ratio of 1.2:1 while...*

After: *As an example, a dog needs a high daily intake of calcium and phosphorus, at a mass ratio of 1.2:1, while...*

3rd paragraph

Removed the text "*In order to evaluate if the health problems in pets may be correlated to the levels of elements in their food, first an orientation is needed to the status of the pet food element content."*

Experimental

1st paragraph

Question: How many samples collect of each brand? Or once a time clear here.

Before: *Ninety-five samples of dry dog food for puppies (n=32) and adults (n=63) of various brands were acquired in the local market of Piracicaba city, São Paulo, Brazil.*

After: *Ninety-five samples of dry dog food for puppies (n=32) and adults (n=63) of thirty-six brands were acquired in the local market of Piracicaba city, São Paulo, Brazil.*

1st paragraph

Question: Why 350 g or how many subsamples made?

A 350 g subsample was taken for preparation because of the capacity of the knife mill.

Before: *After homogenization, a 350 g subsample of each dog food package of 1 kg was reduced to particle size lower...*

After: *After homogenization, one 350 g subsample of each dog food package of 1 kg was reduced to particle size lower...*

1st paragraph

Removed the text "*Dry dog foods were chosen because they are the most common type commercialized in the country."*

1st paragraph

Before: *The powdered material was then homogenized and packaged in ...*

After: *The powdered material was then homogenized and stored in ..*

1st paragraph

The powdered material was then homogenized and stored in bottles of high-density polyethylene for analysis by INAA.

Comment: Give full name first time

Answer: *The full name of the technique (INAA) was mentioned in the last paragraph of introduction, as follows:*

"Instrumental neutron activation analysis (INAA) was used allowing a wide evaluation due to its multi-element capacity (Greenberg et al. 2011)."

2nd paragraph

Question: How many each sample study? It is very important point mention it.

Before: *A test portion of 300 mg of each material was transferred into a high purity polyethylene vial (Posthumus Products, Beverwijk, The Netherlands) for irradiation with neutrons.*

After: *One test portion of 300 mg of each material, from the total 95 samples, was transferred into a high purity polyethylene vial (Posthumus Products, Beverwijk, The Netherlands) for irradiation with neutrons.*

Results and Discussion

1st paragraph

Before: *The minimum and maximum values obtained are shown in Table 1, where one can note the very wide ranges in the fractions*...

After: *The minimum and maximum values obtained are shown in Table 1, where one can note wide ranges of the mass fractions*...

Results and Discussion – Aluminum

1st paragraph

The aluminum results ranged from 48 mg/kg to 11900 mg/kg in adult dog food and from 49 mg/kg to 8500 mg/kg in puppy dog foods.

Comment from reviewer: Repeating units must be omitted in whole text

Answer: We have preferred not to remove the repeating units, since we tried to follow the reference "Guide for the Use of the International System of Units (SI)", proposed by the National Institute of Standards and Technology (2008), which recommend that the units should follow each value in this specific situation.

1st paragraph

Before: *In only one adult food, the aluminum content was below the detection limit (< 21 mg/kg).*

After: *In only one adult food, the aluminum content was below the detection limit, which was 21 mg/kg.*

2nd paragraph

Before*: It was reported before that some additives used in animal feed have aluminum in their composition (Sindirações 2012), e.g., when zeolites...*

After: *It was reported before that some additives used in animal feed have aluminum in their composition (Sindirações 2012), e.g., zeolites...*

3rd paragraph

Before: *... mechanism in the gastrointestinal tract (Berthon, 2002; Codex 2011).*

After: ... *mechanism of the gastrointestinal tract (Berthon, 2002; Codex 2011).*

3rd paragraph

Before: *Aluminum is the most common toxic element found at high levels in dogs, coming mainly from pet food...*

After: *Aluminum is the most common toxic element found at high levels in dogs and its main source is pet food...*

Results and Discussion – Uranium

1st paragraph

Before: *From the total of 95 samples of dog food analyzed, eighteen samples of adult dogs and eight samples of puppies had uranium results above...*

After: *From the total of 95 samples of dog food analyzed, eighteen samples of adult dogs and eight samples of puppies had uranium contents above...*

3rd paragraph

Before: *Their purpose is to adjust the amounts of calcium and...*

After: *Their purpose is to maintain the amounts of calcium and...*

Conclusions

Before: *The mass fractions for most of the twenty elements determined, including As, Cd and Hg, were found at levels that were not considered a concern for dog health.*

After: *The mass fractions for most of the twenty elements determined, including arsenic, cadmium and mercury, were within the permissible limit dog health.*