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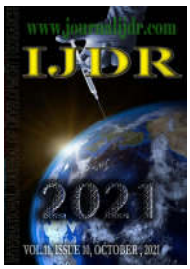

RESEARCH ARTICLE OPEN ACCESS

MICROBIOLOGICAL AND PHYSICO-CHEMICAL QUALITY OF MOIST AND NATURAL PET FOODS SOLD IN BRAZIL

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ABSTRACT

This study evaluated the microbiological and physico-chemical quality of moist and natural foods for dogs and cats sold in Brazil. Three lots of 12 samples were collected, 4 correspond to diets categorized as economic, 2 for each species, 6 as premium, 2 feline and 4 canine, and 2 diets as natural, 1 for each species. The most pet foods analyzed are in accordance with the physico-chemical parameters, except the results of neutral and acid detergent fibre and fat values. All categories of pet foods evaluated comply with the maximum limit defined for coliforms at 45ºC. For mesophilic bacteria was observed counts above the limit in almost all feline foods, except for 1 feline premium brand, this limit was also above in 1 economic and 1 natural canine brand. Only 1 feline premium brand evaluated did not extrapolated the limit of 10^3 CFU/g for molds and yeasts. Indices founds may represent risks to the pet`s health.

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INTRODUCTION

According to the Brazilian Association of the Pet Products Industry (ABINPET), the National Pet Market, which 73.9% corresponds to the pet food industry, registered revenues of R$ 20.3 billion in 2018. In addition, Brazil occupies the 2th place in the world`s pet market (ABINPET, 2019b). Usually, the classification for commercial pet foods is based on the moisture content, which presents the following categories: dry, semi-moist and moist foods. However, in the last years, a new variety of pet foods has been developed; natural and organic foods (Case et al., 2011). Tracking these dietary patterns of pet food, allow not only the evaluate the quality, but also verify the possibility that these diets may have influence in animal`s health. The mycotoxin presence in pet food is related to the fungal growth and therefore temperature and moisture of the product.

The pet food industry faces several challenges related to fungi contamination such as storage conditions, conjugated mycotoxins, grain processing and economic losses. Mycotoxins have been found in final products and raw products of pet food around the globe (LEUNG; DÍAZ-LLANO; SMITH, 2006). The objective of this study was to evaluate the physico-chemical and microbiological quality of commercial moist foods and natural foods, for dogs and cats, sold in the state of Rio de Janeiro, with national distribution.

MATERIALS AND METHODS

Sampling: Samples of infant formula for early childhood were given by the milk bank of the Hospital Universitário Antonio Pedro (HUAP). A total of 36 samples were analyzed, being a total of six batches of two market-recognized brands, assessed on three forms: formula in solid form, collected at the time of opening of the primary

packaging (T1); reconstituted liquid form at the time of sample reconstitution (T2) and the reconstituted liquid form after 24 hours of refrigeration maintenance (T3). The samples were evaluated in duplicates. The solid samples (T1) were collected in sterile centrifuge tubes that were opened at the time of collection and the other samples (T2 and T3) in autoclaved sterilized nipple bottles used by the lactary to offer infant formulas, as well as the other utensils used in the handling and preparation of the products. The samples were prepared following HUAP's operational handling protocols to be able to correctly evaluate the quality of the products offered to patients. The T3 sample was kept refrigerated in the lactary unit itself to evaluate the sample behavior in the unit's storage conditions. The samples were transported to the Laboratório de Controle Microbiológico de Produtos de Origem Animal at Faculdade de Veterinária of Federal Fluminense Universiy, where the bacteriological analysis were performed. Fungi counting, toxins extraction and detection and identification of toxins were performed at the Centro Estadual de Qualidade de Alimentos (CEPQA) at PESAGRO-RJ.

Bacteriological Analyzes: In this work, it was performed the counts of total mesophilic bacteria, *Bacillus cereus*, *Staphylococcus* positive coagulase, lactic acid bacteria, total and thermotolerant coliforms, Enterobacteriaceae counts, and *Salmonella* spp. identification. The inclusion of missing microorganisms in Brazilian standards was decided in awareness of risks that may cause to newborns. Analysis were performed according to the methodologies described in the *Compendium of Methods for the Microbiological Examination of Foods* (APHA, 2015). For the enumeration of the genus *Enterococcus*, the methodology was according to MERCK (2002) and the research of *Cronobactersakazakii* by ISO/TS 22964 method (2006).

Mycological Analyzes: For the fungi count, the serial decimal dilution in plates was performed with inoculation of 0.1mL aliquots of each dilution in two culture media: Dichloran Glycerol Agar (DG18) for xerophilic fungi and Dichloran Bengal Rose Cloranphenicol Agar (DRBC) to estimate the total fungi (PITT and HOCKING, 2009). The plates were incubated at 25°C for seven days. The analysis were performed in analytical duplicates.

AFM1 Detection and Quantification: Samples were extracted using the modified QuEChERS based extraction method following the methodology described in the Association of Official Analytical Chemists (AOAC) Official Methods Manual (AOAC, 2007). All extractions were performed in duplicates. Sample screening was performed using commercial immunoenzymatic kits for AFM1 (Aflatest®, Vicam, Watertown, MA, USA) following the manufacturer's instructions. Quantification and analysis were performed using a VICAM® Series-4EX fluorimeter, according to the manufacturer's guidelines (Watertown, MA, USA). AFM1 standards (5mg) were purchased from Sigma (St. Louis, MO, USA).

Statistical Analysis: Data analysis were performed by analysis of variance (ANOVA). Pearson's correlation and T-test were used to compare enumeration data of different microorganisms in various dairy supplements, as well as the Pearson test in comparing data in mycotoxin contamination in different formulations and comparisons between times. Analyzes were conducted using the computer program PROC GLM in SAS (SAS Institute, Cary, NC).

RESULTS

Considering the tolerance limit for coliforms at 45ºC of 10^3 CFU/g, required by the National Agency for Sanitary Surveillance (ANVISA) for processed meats products destined for human consumption (Brasil, 2001), all categories of pet foods evaluated comply with the maximum limit defined. For mesophilic bacteria, almost all felines foods (6/7) remained above the limit of the technique of 2,5 x 10^2 CFU/g (Brazil, 2003b), except for 1 feline premium brand, this limit was also above in 1 economic brand and 1 natural canine brand, observed in Table 1 and 2.

Only 1 feline premium brand of all pet foods evaluated did not extrapolated the limit of 10^3 CFU/g for molds and yeasts (ABINPET, 2019b). In addition to the high counts, it was also found a wide variety of fungi. Were isolated the genus *Alternaria, Aspergillus, Cladosporium, Fusarium* and *Penicillium*. Among the *Aspergillus* genus was found the species *A. flavus,A. fumigatus*, *A. ochraceus, A. oryzae, A. parasiticus* and *A. niger*, among the *Penicillium* genus, species *P. citrinum and P. citreonigrum*, and the *Fusarium* species and *F. verticillioides* and *F. chlamydosporum*. The majority of samples were contaminated by at least one mycotoxin, 83,3% (10/12). The study indicates the *Fusarium* spp. mycotoxins DON (≤945 ppb), ZEN (≤800 ppb) and FB (≤750 ppb) was the contaminants most prevalent and Aflatoxins was not detected in the samples. But all samples are below the LOQ of techniques. These results are in accordance with Maia and Siqueira (2002) and Campos et.al. (2008), who found low incidence rate of Aflatoxin contamination in Brazilian dog and cat food. As for moisture (Table 3) the average of all categories evaluated complies with the maximum limit of 84% allowed for moist foods and have protein levels above the minimum established by Ministry of Agriculture, Livestock and Supply (MAPA) (Brasil, 2003a). Most of the fat values comply with the legislation (Brasil, 2003a), with exception of 1 economic and 1 premium canine brands that were under minimum of 1.0%, also 1 economic and 1 premium feline brands that were under minimum of 1.5%. The average of all the samples exceeded the limit recommended by MAPA for NDF (Brasil, 2003a). The analyzed samples respected the maximum value of 2.5% for ashes (Brasil, 2003a). The pH values observed ranged from 5 to 7. In the TVBN analysis are determined total volatile basic nitrogen compounds resulting from the autolytic and microbial enzymatic action on muscle proteins, besides other substances, whose quantities vary with the storage time of the product, increasing as the deterioration advances. All the results were under the limit of 0.3 mg/kg established by Regulation and Industrial and Sanitary Inspection of Products of Animal Origin (RIISPOA) (Brasil, 2017).

DISCUSSION

Bacterial Evaluation: Pet food production manufacturers mainly apply high heating processes, moist foods undergo a commercial sterilization, while natural foods do not undergo this type of processing, contain predominantly fresh ingredients, and therefore require greater care during the food handling. The high temperature is the most relevant influence in microorganism contaminant´s reduction, however, the effectiveness of these processes depends on conditions used, like temperature, duration of the process or humidity (Witaszak et al., 2020). This reflects the higher contamination of mesophiles found in the natural foods, but even pet foods that comply standards, reached close or at the limit of the technique, which also indicates a low efficiency of the processing of these pet foods. The samples contain significant toxigenic mycobiota contaminants, as *Fusarium* spp. and *Aspergillus* spp. Some of those strains are able in mycotoxins production. Detected levels of DON, ZEN, and FBs were much below Food and Drug Administration (FDA) and EU recommendations but in this particular case it does not guarantee safety, there are no quality control parameters for these microorganisms in the Brazilian legislation at the moment. The Brazilian pet foods industries are subject only to a recommendation from ABINPET (2019b), an unofficial body, which is based on what is defined by European Union (EU) Regulation No. 142 of 2011, that is not mandatory in Brazil. The presence of fungi can become a health hazard of animals causing infection and toxinfection since some strains may produce mycotoxins. In dogs and cats, the effects of mycotoxins are severe and can lead to death, loss of nutrients, altered organoleptic properties and reduced validity of the pet food (Campos, 2007). Our results were in accordance with the data obtained during international research on mycotoxins in feed (Witaszak et al., 2020). These samples contain significant toxigenic mycobiota contaminants, such as *Fusarium* spp. and *Aspergillus* spp. Some of those strains are able in mycotoxins production. Detected levels of DON, ZEN, and FBs were much below Food and Drug Administration (FDA) and EU

Table 1. Average and Standard Deviation of Microbiological Counts in Samples of Feline Moist and Natural Foods in Colony Forming Unit Per Gram (CFU/g; n=36)

Samples	Microbial load (CFU/g)			
	Coliforms at 45°C	Total Coliforms	Aerobic Mesophilic	Yeasts and Molds
Feline Economic Brand 1	$\geq 1.20 \times 10^2$	$3.80 \times 10^2 \pm 2.12 \times 10^2$	$1.79 \times 10^3 \pm 1.95 \times 10^3$	$5.38 \times 10^4 \pm 6.80 \times 10^4$
Feline Economic Brand 2	$\geq 1.20 \times 10^2$	$\geq 1.20 \times 10^2$	$6.25 \times 10^2 \pm 3.08 \times 10^2$	$1.68 \times 10^4 \pm 1.64 \times 10^4$
Feline Premium Brand 1	$\geq 1.20 \times 10^2$	$2.44 \times 10^2 \pm 1.01 \times 10^2$	$2.01 \times 10^3 \pm 2.82 \times 10^3$	$3.00 \times 10^4 \pm 4.24 \times 10^4$
Feline Premium Brand 2	$\geq 1.20 \times 10^2$	$\geq 1.20 \times 10^2$	$3.40 \times 10^2 \pm 4.67 \times 10^2$	$7.20 \times 10^2 \pm 6.10 \times 10^2$
Feline Premium Brand 3	$\geq 1.20 \times 10^2$	$\geq 1.20 \times 10^2$	$6.70 \times 10^2 \pm 4.67 \times 10^2$	$2.56 \times 10^4 \pm 3.45 \times 10^4$
Feline Premium Brand 4	$\geq 1.20 \times 10^2$	$\geq 1.20 \times 10^2$	1.00×10^1	$1.28 \times 10^4 \pm 5.66 \times 10^3$
Feline Natural	$1.84 \times 10^2 \pm 5.23 \times 10^1$	4.40×10^4	$1.41 \times 10^5 \pm 1.03 \times 10^5$	$5.27 \times 10^4 \pm 6.89 \times 10^4$

Note. Values represent the average of microbial load by colony forming units per gram of 3 different lots collected during the study period. P>0.05.

Table 2. Average and Standard Deviation of Microbiological Counts in Samples of Canine Moist and Natural Foods in Colony Forming Unit Per Gram (CFU/g; n=36)

Samples	Microbial load (CFU/g)			
	Coliforms at 45°C	Total Coliforms	Aerobic Mesophilic	Yeasts and Molds
Canine Economic Brand 1	$\geq 1.20 \times 10^2$	$2.44 \times 10^2 \pm 1.01 \times 10^1$	$2.11 \times 10^4 \pm 2.96 \times 10^4$	$1.84 \times 10^3 \pm 1.09 \times 10^3$
Canine Economic Brand 2	$\geq 1.20 \times 10^2$	$\geq 1.20 \times 10^2$	1.00×10^1	$2.65 \times 10^3 \pm 3.53 \times 10^3$
Canine Premium Brand 1	$\geq 1.20 \times 10^2$	$\geq 1.20 \times 10^2$	1.00×10^1	$2.17 \times 10^3 \pm 2.09 \times 10^3$
Canine Premium Brand 2	$\geq 1.20 \times 10^2$	$3.60 \times 10^2 \pm 1.96 \times 10^2$	$1.67 \times 10^1 \pm 9{,}43$	$2.70 \times 10^3 \pm 2.33 \times 10^3$
Canine Natural	$\geq 1.20 \times 10^2$	4.40×10^4	$1.26 \times 10^4 \pm 1.03 \times 10^4$	$1.35 \times 10^4 \pm 1.87 \times 10^4$

Note. Values represent the average of microbial load by colony forming units per gram of 3 different lots collected during the study period.P>0.05.

Table 3. Average and Standard Deviation of the Lots by Pet Food Classification of the Centesimal Composition of Moist and Natural Pet Foods Samples in Percentage of Dry Matter (n = 36)

Pet Food Classification	Moisture	Crude Protein	Fat	Ash	NDF
Feline Economic	82.67% ± 3.01 [a]	7.60% ± 1.89 [a]	0.73% ± 0.36 [a]	1.45% ± 0.43 [a]	5.40% ± 1.82 [a]
Feline Premium	79.76% ± 2.97 [a]	10.49% ± 2.05 [a]	1.09% ± 0.98 [a]	1.38% ± 0.22 [a]	7.02% ± 4.58 [a]
Feline Natural	74.17% ± 3.72 [a]	18.40% ± 2.71 [b]	3.15% ± 1.35 [a]	1.67% ± 0.90 [a]	18.70% ± 5.88 [b]
Canine Economic	82.38% ± 2.48 [a]	7.37% ± 2.11 [a]	0.43% ± 0.14 [a]	1.15% ± 0.23 [a]	7.48% ± 3.51 [a]
Canine Premium	81.72% ± 2.71 [a]	9.12% ± 1.46 [a]	0.70% ± 0.25 [a]	1.40% ± 0.23 [a]	14.02% ± 9.68 [a]
Canine Natural	75.43% ± 0.87 [a]	9.80% ± 2.74 [a]	2.50% ± 0.71 [a]	0.87% ± 0.31 [a]	12.86% ± 1.96 [a]

Note. ANOVA comparative fator. a, b Represent difference of means in the evaluated column, when there is a difference between letters follow the significance of ($P \leq 0.05$).

recommendations and Aflatoxins was not detected in the samples, but in this particular case it does not guarantee safety. This suggests that the issue also covers other pet food products and, hence, research should be extended to include other types of pet food. The centesimal composition of the pet foods may negatively affect the health of the animals if they do not provide the adequate amount of nutrients. It should be noted that it is a consensus among pet food buyers that the level of protein is what guarantees the quality of the product. However, what many consumers are unaware is that not always the highest levels of protein guarantee the greatest availability of essential amino acids (Melo et al., 2014). Unfortunately, the levels of essential amino acids are not a required parameter in pet food labels in Brazil, what limits the evaluation of the quality of the protein source by the ingredients used. It`s recommended that pet food quality standard (economic, premium, super premium) became mandatory on the label, following parameters established by the legislation. Dietary fibers have gained renewed interest in the pet food industry, as they play an important role in modulating bowel movement, influencing immune function and gut microbiota profile, also so diluting caloric density (Godoy et al., 2013). However, it`s recommended caution when supplementing with large amounts, as dietary fiber can mildly alter nutrient digestibility. Fat may increase the caloric intake, and in some cases lead the animal to the development of obesity (Brunetto et al., 2011). Low levels of fat were observed in some of moist foods samples analyzed, so the use of these foods can be indicated in the management of obesity, but it must attend to the minimum levels of fat, because fat provide essential fatty acids and allow the absorption of fat-soluble vitamins. These samples contain significant toxigenic mycobiota contaminants, as *Fusarium spp. and Aspergillus spp.* some of those strains are able in mycotoxins production. Detected levels of DON, ZEN, and FBs were much below Food and Drug Administration (FDA) and EU recommendations but in this particular case it does not guarantee safety. This suggests that the issue covers also other pet food products and, hence, research should be extended to include other types of pet food. The most pet foods analyzed are in accordance with the physico-chemical parameters required by MAPA, except for fiber and fat values. Most pet foods analyzed were not in accordance with the fungal parameters suggested by ABINPET. The ingestion of pet food contaminated by fungi and its metabolites may negatively affect the health of pets, however, the samples had overall acceptable mycotoxicological quality even though the Brazilian legislation regarding the microbiological control of animal feed does not determine accurate limits of fungal and mycotoxins contamination. Further research of the quality of pet food in the Brazilian market and the implementation of fungi and mycotoxin contamination legislation is advised in order to protect the companion animals from the harmful effects of those contaminations The ingestion of food contaminated by bacteria, fungi and metabolites may negatively affect the health of pets, however, the Brazilian legislation regarding the microbiological control of animal feed does not determine accurate limits of contamination.

CONCLUSION

The products analyzed are in accordance with Brazilian legislation and current international regulations, being considered safe for consumption, but the need for immediate consumption after resuspension and adequate storage after opening the package is recommended to avoid microbiological development. Any hour after that period confers risk for consumers. Although the products analyzed are in compliance, the identified contaminations are indicative of the need for greater efforts in the control of the manufacturing process, whether the raw material quality or the equipment's cleaning processes. The constant review of professional training and hygiene protocols used in lactary units are also important to minimize the risks to consumers.

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