

MICROBIOLOGICAL QUALITY OF DOG FEED SOLD IN SEALED PACKAGES AND IN BULK.

(QUALIDADE MICROBIOLÓGICA DE RAÇÕES PARA CÃES COMERCIALIZADAS NO VAREJO EM EMBALAGEM FECHADA E A GRANEL)

T. M. S. GIRIO¹, A. NADER FILHO², O. D. ROSSI JUNIOR²,
L. A. AMARAL², R. J. S. GIRIO²

SUMMARY

In recent years, the segment of pet food has had a significant growth in sales volume in Brazil. The Brazilian market for dog feed is growing at the highest rate worldwide. The present study analyzes 15 brands of dog food regarding their hygienic-sanitary quality, sold either in sealed packages or in bulk. Samples were obtained from specialized shops in Jaboticabal, SP, Brazil. Most Probable Number of total and thermotolerant coliform bacteria, counting of mold and yeasts, mesophilic microorganisms and bacteria of the genus *Salmonella* were also determined. Results show that no significant difference was detected in counting of molds, yeasts and mesophilic micro-organisms among the commercialized samples either in sealed packages or in bulk. Higher percentages of samples contaminated by molds and yeasts and higher fecal pollution contamination indexes were detected in bulk feed samples. Bacteria of genus *Salmonella* were not isolated in the analyzed samples. It is recommended that the bulk feed be packed and sealed for commercialization, so that its micro-biological quality is preserved.

KEY-WORDS: Animal feed. Dog. Microbiology.

RESUMO

Nos últimos anos, o segmento de alimentos de animais de estimação no Brasil vem apresentando um expressivo crescimento no volume de vendas. O mercado brasileiro de rações para cães apresenta os maiores índices de crescimento mundial. Com os objetivos para verificar a qualidade higiênico-sanitária e a influência da venda a granel foram analisadas 15 marcas de rações para cães, oriundas de embalagens fechadas e da comercialização a granel. As amostras foram obtidas no comércio varejista do Município de Jaboticabal/SP. Foram realizadas as determinações dos números mais prováveis de coliformes totais e termotolerantes, contagens de bolores e leveduras microrganismos mesófilos e a pesquisa de bactérias do gênero *Salmonella*. Os resultados obtidos evidenciaram que não houve diferença significativa nas contagens de bolores e leveduras e microrganismos mesófilos entre as amostras comercializadas em embalagens fechadas e a granel. Entretanto, nas amostras de rações comercializadas a granel foram verificadas maiores porcentagens de amostras contaminadas por bolores e leveduras e uma maior contaminação por indicadores de poluição fecal. Não foram isoladas bactérias do gênero *Salmonella* nas amostras analisadas. Sugere-se que na comercialização a granel a ração seja embalada para com isso preservar a qualidade microbiológica do produto.

PALAVRAS-CHAVE: Ração animal. Cães. Microbiologia.

¹ Uzinas Químicas Brasileira – Saúde Animal. * Corresponding author: thais@ucbvvet.com.br

² Departamento de Medicina Veterinária Preventiva e Reprodução Animal, Faculdade de Ciências Agrárias e Veterinárias, Unesp, Jaboticabal, SP.

INTRODUCTION

In the flowchart of dog and cat food production, Butolo (2002) states that when the ingredients are received in the factory, samples should be taken to determine their physico-chemical and microbiological properties. To ensure food quality, the storage of the raw material, primarily in bulk, must be well controlled, since humidity, insects, rodents and storage time can compromise the quality of the raw material. The same precautions should be taken regarding the final product in the shops where it is sold.

According to Andrade & Nascimento (2005) one of the risk factors to animal health is food contamination by fungi and other microorganisms. This contamination can occur during the production process or storage of the raw material of animal origin or grains, which are widely used in food production of several animal species, or even during industrialization and packaging of the final product (SILVA, 1998).

An important factor that can compromise the quality of the feed is the exposure to agents that carry the microorganisms, as it can be seen in several different works. The multiplication of *Salmonella typhimurium* has been reported in cockroaches (*Periplaneta americana*) by Klowden & Greenberg (1976). Kopanic et al. (1994) observed that cockroaches contaminated with *Salmonella* spp. can contaminate other cockroaches and the food they come in contact with, thus evidencing the potential of these insects in the transmission of pathogenic microorganisms. *Salmonella* spp. was isolated in 6% of rats, 10% of mice, 1% of the cockroaches and 7% of ants that were examined by Singhi et al. (1980). Hilton et al. (2002) found *Salmonella* spp. in 8% of stool samples of rats (*Rattus norvegicus*) and reported that the *Salmonella* remained viable in the feces of rodents for up to 86 days.

Santos et al. (2000) concluded that meat and bone meal contaminated by *Salmonella* were the main source of pathogens in the animal feeds. The counts of mesophilic bacteria, molds and yeasts were not effective indicators. The presence of fecal coliform in the feed and ingredients is associated with lack of general hygiene during product handling and storage.

Varley (2003) reported the presence of *Aspergillus* spp. and *Penicillium* spp. in sealed and open packages that presented a small degree of moisture. The author also stated that this genus is important since it produces mycotoxins known as aflatoxins and ochratoxins that are highly toxic to dogs. These mycotoxins according to Mallman et al. (2002) may cause in dogs and humans clinical signs such as, loss of appetite, diarrhea, vomit and hemorrhage, as well as hepatotoxicity and nephrotoxicity, in addition to a carcinogenic effect.

Bernardi & Nascimento (2005) reported that the feed contamination by fungi may happen through the environment and its development may be favored by humidity, temperature and substrate with the multiplication of the microorganisms and production of toxic metabolites, like mycotoxins.

A study by Andrade & Nascimento (2005) conducted in Pelotas, RS, to determine the presence of filamentous fungi in commercial dog food, verified the presence of a larger number of fungi (UFC/g) in bulk feed kept in the open. In all the examined samples, the fungi *Aspergillus* spp. and *Penicillium* spp were present.

The hygienic quality of the feed is assessed by the presence of indicator organisms with emphasis on the group of thermotolerant coliform, in the case of feed, the presence of salmonella (SANTOS et al., 2000), the exposition of the feed to a compromising environment (HINTON & MEAD, 1992) and its shelf life (SILVA et al., 1997).

The objective of this study is to determine the microbiological quality of commercial feeds sold either in bulk or sealed packed, and to establish if bulk sales can depreciate its hygienic-sanitary quality.

MATERIAL AND METHODS

Sampling

We collected samples of 15 commercially available brands of dog feed in the cities of Jaboticabal and Ribeirão Preto, in 2006. From each brand, 2 samples were taken from sealed packages and 2 samples of 1 kg of the same brand that is commercially available in bulk, a total of 60 samples. We tried to collect samples from feed that originated from the same manufacturing lot.

Microbiological analysis

Sample preparation and dilution

The first dilution 10^{-1} was obtained by diluting 25 g of feed in 225 mL of peptone water 0.1%. From this dilution, consecutive decimal dilutions were prepared by transferring 1 mL of the previous dilution to 9 mL of diluent.

The most probable number (MPN) of total and thermotolerant coliform, as well as mold, yeast and mesophilic microorganism counts and *Salmonella* sp presence were determined according to the Compendium of Methods for the Microbiological Examination of Foods (APHA, 2001).

Statistical analysis

The number of contaminated feed samples that originated from either bulk or sealed packaged were compared by Chi-square test ($p < 0.05$) (STELL & TORRIE, 1960).

RESULTS AND DISCUSSION

Table 1 shows that commercially available food sold in bulk was more contaminated compared to the one sold in sealed packaging. From the bulk feed

Table 1 – Number and percentage of feed samples, commercially available in bulk or sealed packaging, contaminated by total (TC) and thermotolerant (TheC) coliform, molds and yeasts (MY), Jaboticabal, 2006.

Type	TC	TheC	MY	Total
Sealed	3(10%)A	2(6.7%) A	12 (40%)A	17(56.6%)
Bulk	2(6.7%)A	2(6.7%) A	16(53.3%)A	20(66.6%)
Total	5(16.7%)	4(13.4%)	28(93.3%)	37(66.6%)

Values followed by the same letters in the column do not differ ($p > 0.05$).

samples, 66.6% were contaminated, 10% with total coliform, 6.7% with thermotolerant coliform and 53.3% with molds and yeasts.

From the results, it can be seen that the major contaminants found in the feeds were molds and yeasts. A result also reported by Andrade & Nascimento (2005) who observed that contamination by these agents can occur during any step of the manufacturing process. Bulk feeds had a higher percentage of samples contaminated by molds and yeasts compared to sealed packaging feed.

A possible explanation for the contamination of food stored and sold in bulk is given by Bernardi & Nascimento (2005) who stated that constant contact with air and during handling as well, enable fungi such as *Penicillium* spp., *Aspergillus* spp., *Rhizopus* spp. and *Fusarium* spp present in the environment to be transferred to the feed. These are airborne agents can easily colonize the food, especially when humidity and temperature are favorable. However, moisture contamination of sealed packaged food can also happen during storage in the factories, transportation or retail commercialization. The feed contaminated with molds and yeasts, depending on the contaminating fungi, can pose serious health threat to both, animal and handler (BERNARDI & NASCIMENTO, 2005). Mallman et al. (2002) reported that mycotoxins can cause in dogs and humans clinical signs such as loss of appetite, diarrhea, vomit, hemorrhage, as well as as hepatotoxicity and nephrotoxicity, in addition to carcinogenic effect.

A study conducted by Varley (2003) found that *Aspergillus* spp. and *Penicillium* spp were present in either bulk or sealed packaged food that had small degree of moisture. The author stated that these genus are important because they produce mycotoxins known as aflatoxins and ochratoxins that are highly toxic for dogs.

The results showed that MPN of thermotolerant microorganisms was higher in the bulk feed, the count was higher than 10^2 microorganisms per gram of feed.

The MPN, 3 – 100 microorganisms per gram, was found in 7% and 3% of sealed packaged and bulk food, respectively. The percentage of the samples with MPN lower than 3 microorganisms per gram, was practically the same for both types of commercialization. These results show that the quality of the food sold in bulk or sealed packaged had a satisfactory hygienic-sanitary standard. Probably the manufacturing process of the finished product makes difficult the contamination by environmental bacteria. Even though dog feeds are rich in proteins, water activity (A_w) is low, which limits the growth of microorganisms. However, one can not disregard the possibility of contamination of open containers used for bulk sales by fecal coliform from insects or rodents (FRAZIER & WESTHOFF, 1993).

The average numbers of mesophilic microorganisms found in the food samples were higher in the food sold in bulk (Table 2). It is also seen that percentage of the foods containing more than 10^4 UFC/g of mesophilic microorganisms was higher for bulk food compared to sealed package. According to the microbiological criteria for food meal adopted in the Netherlands, as cited by Andriquetto et al. (1990), the feeds in the present study are considered good quality regarding the mesophilic counts ($< 10^6$ UFC/g).

Low counts of mesophilic bacteria in diets prepared manually using raw material of vegetal origin were found by Gonçalves et al. (2005) in samples collected immediately after the mixing process. The greater number of mesophilic bacteria in bulk feed can be explained by the longer exposure time of the product to the environment. The presence of mesophilic bacteria in the food at acceptable levels ($< 10^6$ UFC/g) does not pose health threat to the animals as reported by Santos et al. (2000).

Table 2 – Percent distribution of contaminated food samples, in bulk or sealed packaged, according to molds and yeasts, thermotolerant coliform and mesophilic microorganisms, Jaboticabal, 2006.

MICROORGANISMS	TYPE	
	SEALED	BULK
Molds and yeasts (UFC/g)	77%	83%
0 — 100	20%	17%
100— 1,000 > 1,000	3%	ND
Thermotolerant coliform (NMP/g)		
< 3	93%	94%
3-100	7.0%	3.0%
> 100	ND	3.0%
Mesophilic microorganisms (UFC/g)		
0 I—100	28.6%	38.5%
100 I—10,000	43.8%	26.0%
10,000 I— 100,000	28.6%	38.5%

ND- not detected in any sample

In this study, microorganisms of the genus *Salmonella* were not present in food, either in bulk or sealed packaged. However, the concern remains that food sold in bulk may contain this bacteria or other pathogens, since it remains exposed to the environment and contaminant agents such as insects and rodents. Kopanic et al. (1994) and Hilton et al. (2002) proved that this possibility exists when there is little concern about the hygienic-sanitary conditions of the establishments that sell animal feed.

The high percentage of bulk food samples contaminated by yeasts and molds, and the large population of microorganisms indicating fecal pollution, shows the need of packaging the food in order to avoid contact with the environment and with animals that may act as mechanical vectors of spoilage and/or pathogenic agents.

CONCLUSIONS

It can be concluded that both types of dog feeds, in bulk and sealed packages, were contaminated by total and thermotolerant coliform, mesophilic microorganisms, as well as molds and yeasts. The lack of microbiological standards makes it difficult to evaluate the potential risk that these foods pose to

animal health. The results suggest the need for packaging the food in order to preserve its quality.

ACKNOWLEDGMENTS

The authors thank FAPESP for financial support.

REFERENCES

- APHA. American Public Health Association. **Compendium of methods for the microbiological examination of foods**. 4ed. 676 p. 2001.
- ANDRADE, R. M.; NASCIMENTO, J. S. Presença de fungos filamentosos em ração para cães comercializadas na cidade de Pelotas – RS. **Arquivo do Instituto Biológico de São Paulo**, São Paulo, v.72, n.2, p.10-12, 2005.
- ANDRIGUETO, J. M.; MINARDI, I.; GEMAEL, A.; FLEMMING, J. S.; SOUZA, G. A.; BONA FILHO, A. **As bases e os fundamentos da nutrição animal**. 4 ed. São Paulo: Nobel, 1990. 396p.

- BERNARDI, E.; NASCIMENTO, J. S. Fungos Anemófilos na praia do Laranjal, Pelotas, Rio Grande do Sul, Brasil. **Arquivo do Instituto Biológico de São Paulo**, São Paulo, v.72, n.1, p.93-97, 2005.
- BUTOLO, J. E. **Qualidade de ingredientes na alimentação animal**. Campinas: Colégio Brasileiro de Nutrição Animal, 2002.
- FRAZIER, W. C.; WESTHOFF, P. C. **Microbiologia de los alimentos**. 4 ed. Zaragoza: Acribia, 1993. 229p.
- GONÇALVES, C. M.; LOPES, K. G. S.; CREPALDI, I. C.; GOES NETO, A.; UETANABARO, A. P. Ocorrência de microrganismos em ração animal preparada artesanalmente a partir do Licuri (*Syagrus coronata*). **Sitientibus Série Ciências Biológicas**, Feira de Santana, v.5, n.2, p.53-55, 2005.
- HILTON, A. C.; RICHARD J.; WILLIS, R. J.; HICKIE S. J. Isolation of *Salmonella* from urban wild brown rats (*Rattus norvegicus*) in the West Midlands, UK. **International Journal of Environmental Health Research**, v.12, n.2, p.163-168, 2002.
- HINTON, M.; MEAD, G. C. Bacterial pathogens in animal feed and their control. **World's Poultry Science Journal**, London, v.48, n.1, p.72-73, 1992.
- KLOWDEN, M.; GRENNBERG, B. *Salmonella* in the American cockroach: evaluation of vector potential through dosed feeding experiments. **Journal of Hygiene**, Londres, v.77, n.1, p.105-111, 1976.
- KOPANIC JR, R. J.; SHELDON, B. W.; WRIGHT, C. G. Cockroaches as vectors of Salmonella: Laboratory and field trials. **Journal of Food Protection**, v.57, n.2, p.125-132, 1994.
- MALLMAN, C.A.; MÜRMAN, L.; KOWALSKI, C. H.; SILVEIRA, V. G. Níveis de contaminação por aflatoxinas em rações animais. CONGRESSO BRASILEIRO DE MEDICINA VETERINÁRIA, XXIV, Gramado-RS, 2002. **Anais...**Gramado-RS.
- SANTOS, E. J., CARVALHO, E. P., SANCHES, R. L., BARROS, B. E. B. Qualidade microbiológica de farinhas de carne e ossos produzidas no Estado de Minas Gerais para produção de ração animal. **Ciência Agropecuária**, Lavras, v.24, n.2, p.425-433, 2000.
- SILVA, L. O. N. **Sistema de qualidade (NB 9000) em fábricas de rações**. 1998. 205f. Tese (Doutorado) – Faculdade de Engenharia Agrícola, Universidade Estadual de Campinas, Campinas.
- SILVA, N.; JUNQUEIRA, V. C. A.; SILVEIRA, N. F. A. **Manual de métodos de análise microbiológica de alimentos**. São: Varela, 1997. 87p.
- SINGHI, S. P.; SETHI, M. S.; SHARMA, V. D. The occurrence of salmonellae in rodent, shrew, cockroach and ant. **International Journal of Zoonoses**, v.7, n.1, p.58-61, 1980.
- STEEL, R. G. D.; TORRIE, J. H. **Principles and procedures of statistics**. New York: Mc Graw, 1960. 481p.
- VARLEY, M. Where are we now with control of mycotoxins? **Pig Progress:the international magazine on pig production**, The Netherlands, v.20, n.10, p.24-25, 2003.