

INFORMATION ON THE PRESENCE OF 2- ALKYL CYCLOBUTANONES IN ANIMAL FEED AFTER IONIZING RADIATION TREATMENT

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ABSTRACT

In agribusiness, it is related to the development of the activities of creation, production and commercialization of pets in the Pet segment. This market grew 7.6% between 2014/2015, generated R\$ 18 million in Brazil and 67.3% of this value refers to products for animal feed, commonly called pet food. In animal feed, the raw materials are chosen according to the method (s) of processing (s) in which the product is submitted, its stability in the process and its nutritional source during the validity of the product. In food preservation, the irradiation process is a treatment that seeks to reduce the microbial load of foods, however, it can also alter the composition of the present ingredients and form radiolytic products in the formulation that are still under study. In products containing the presence of fat, the main concern is 2-Alkylcyclobutanones (2-ACBs), which are radiolytic products formed exclusively post-processing with ionizing radiation. The formation of 2-ACBs is directly related to the lipid concentration and the dose of irradiation. The objective of this work is to describe the possible radiolytic by-products formed in canine rations containing extruded fat after irradiation and to determine if the formation of 2-ACBs depends on the increase of the radiation dose and, as a consequence, verify their cytotoxicity and genotoxicity.

Keywords: Food irradiation, pet food, 2-ACBs, 2-alkylcyclobutanones and irradiated ration.

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1.INTRODUÇÃO

In agribusiness it is related to the development of the activities of creation, production and commercialization of pets the pet segment. This sector is divided into Pet Food (dry, moist and snack foods), Pet Care (equipment, accessories, and hygiene and beauty products), Pet Vet (Veterinary Medicines) and ingredients. The pet population in Brazil is 132.4 million, being the 4th largest in the world where 360.8 million of this total are dogs. This market grew 7.6% between 2014/2015, earned R\$ 18 million and 67.3% of this amount refers to products for animal feed, commonly called pet food¹.

In the world dogs are the main animals chosen to be pets, this happens among other factors, by the transition from the role of the dog in the society of utility to social, in that passage the dog moved away from its carnivorous feeding of its wild ancestors to adopt food supplied by man². Currently, there are 360.8 million dogs in the world, with 52.2 million dogs in Brazil alone¹. As a result of this population the products destined for this market are varied and have wide expansion, however, processed food is the area of greatest volume and investment³.

2. CANINE NUTRITION

In animal nutrition the food may be a single ingredient or an elaborate formulation that may have up to 60 items in its composition. The raw materials are chosen according to the process method (s) in which the product is submitted its stability in the process and its nutritional source during the validity of the product. In product development every ingredient has a purpose and provides some nutritional benefit. Additives are categorized into proteins, carbohydrates, fiber, fat, vitamins and minerals. They are selected considering the following aspects: availability, nutrient levels, functionality, palatability, digestibility, cost and safety⁴.

The specific needs of each stage of life, the physiological condition, lifestyle and the various pathophysiological conditions of animals have since become relevant topics in the research for formulating new rations in which the adequacy of the nutritional composition, the matrix of the ingredients and Their processing must follow varied parameters⁵.

The composition of the animal diet varies according to the nutritional biochemistry of the species for which it is intended. According to Caulfield *et al* 2008 after the irradiation process there is reduction of vitamin A besides possible changes in important constituents of the formulation in rations. This work specifically focuses attention on the changes that occur with fats, since after irradiation, in the literature we find alerts of formed radiolytic products that may present some toxicity both in food for humans and animals.

The quality of the fat is important for canine health, since this nutrient assists in the hepatic functioning, beauty of the hair, reproduction among other functions acts as a contribution of essential fatty acids, they are still palatabilizantes and an important energetic fraction of the rations, the main sources Of this ingredient in canine feeds are beef tallow, chicken fat, corn oil, soybean and sunflower ^{6,3,2}.

3.CONTAMINANTS IN CANINE FEEDS

The main contaminants found in foods for these animals come mainly from the diversity of raw materials that are part of the composition of this food. This contamination often occurs due to the inadequate handling and low quality of these ingredients. The biological contaminants commonly found in these foods are part of several groups of microorganisms (bacteria, fungi, protozoa and viruses), as well as their metabolites and toxins including bacterial toxins, mycotoxins and biogenic amines ^{7,8}. Severe cases of infection in pets may be caused by biological contamination present in their food⁸.

Due to their nutritional losses, fungi produce mycotoxins on substrates such as corn, wheat, soybeans and rice, which are ideal for their growth and are normally used as ingredients in extruded diets. Among the types of toxigenic fungi (mycotoxin producers), the fungi of the genus *Alternaria*, *Cladosporium*, *Fusarium*, *Aspergillus* and *Penicillium* predominate ^{8,9,10}. Liver, digestive tract, kidneys, reproductive system and central system are some of the targets of these mycotoxin-producing fungi as well as exert effects on immunity and blood coagulation of animals⁷.

In the final product the effects of mycotoxins in addition to nutritional loss cause changes in organoleptic properties and decrease in shelf life. In dogs it has severe effects that can lead to death, since this type of animal, specifically when exposed to this contaminant regularly, due to its sensitivity to acute hepatotoxic effects, may have chronic liver damage, as a consequence of this the amount of aflatoxins present In animal feed is a quality criterion ^{7,10}.

4.CONSERVATION OF FOOD

Food preservation is the process of ensuring the physical, chemical and biological stability of food even in conditions where this would not be feasible. This process does not revert any deterioration that has already begun, it may delay it, so the food to be conserved must reach the process with good quality¹¹. Dangerous microorganisms contribute to chemical changes in food, these changes in lipids are Caused by bacteria that produce specific enzymes and break their

structure causing the appearance of fatty acids, smaller compounds that are responsible, in this case, for odors unpleasant to food. Food irradiation is one among several existing technologies that is recognized with a safe and effective method for preserving a wide range of foods ^{12,13,14,15}.

Irradiation treatment is widely applied in the food industry in inhibiting budding in bulbs and tubers, delaying maturation and senescence in fruits and vegetables, reducing microbial load in meat, fruits and vegetables, eliminating parasites and pests in grains, cereals, Rations, fruits and spices and in ready-to-eat foods preserved at room temperature. Many foods need to be irradiated already packed, mainly so that there is no recontamination after procedure, so the nature of the packaging and its interaction with the irradiation should be considered¹⁴.

Regardless of the purpose in which it is desired to use the treatment by irradiation it is necessary that the safety of its application is previously analyzed. What ensures the application in this type of process is the dose at which it will be submitted. Such doses are minutely controlled and should be investigated according to the time of exposure, the type of food to be subjected in order to achieve the goal with minimal impairment to the overall integrity of the food ¹⁶. However, like any process to which The food is treated there is always some loss. Studies have identified the formation of radiolytic products, especially free radicals after irradiation in foods containing fat ^{17,18,19}.

The production of free radicals responsible for nutritional and sensory alterations in the food is induced in lipid irradiation, which react with oxygen, leading to the formation of carbonyls ²⁰ (Brito et al, 2002). In systems based on HACCP (Hazard Analysis and Critical Control Points) the use of ionizing radiation combined with other food preservation procedures is advantageous ^{21,22}. However, the greatest concern in the use of this method and the formation of radiolytic compounds ²¹.

Different doses of irradiation promote different functions. To reduce deterioration caused by pathogenic organisms in refrigerated foods, doses ranging from 1 to 7 kGy are usually applied, increasing storage time from 1 to 3 kGy, to retard the ripening process in fresh fruits and vegetables from 0.25 to 1, 0 kGy, insect disinfestation (phytosanitary) doses up to 1 kGy, parasites 2 to 5 kGy, inhibition and / or germination of bulbs and tubers 15 to 70 Gy ^{23, 24, 25, 26, 27}.

Foods containing fat, when treated by accelerated electron beam, x-ray or rad radiation (60Co or 137Cs) promote the radiolysis in triglycerides with the consequent formation of 2-alkylcyclobutanones (2-ACBs). These molecules are cyclic compounds with four carbons and have in total the same number of carbons of the lipid that generated it ²⁴, ie, knowing the triglyceride of origin it is possible to predict the 2-ACB that will be formed ²⁸. The formation of 2-ACBs are directly related to the lipid concentration and the dose of radiation to which the triglyceride is subjected. ^{21, 28, 29, 30}.

According to the triglyceride present in the food we have the formation of different 2-alkylcyclobutanones ^{28,29}. 2-ACBs are not found in unirradiated foods ²¹. 2-dodecylcyclobutanone (DCB) is formed from palmitic acid C16; 2-tetradecylcyclobutanone (2-TCB) is derived from C 18 stearic acid. The following table shows the main fatty acids and the respective irradiation products formed therefrom.

Fatty Acid	Number of Carbs	Irradiation Product
Capric Acid	C10:0	2-Hexylcyclobutanone (2-HCB)
Lactic Acid	C12:0	2-Octylcyclobutanone (2-OCB)
Myristic Acid	C14:0	2-Decylcyclobutanone (2-DCB)
Palmitic Acid	C16:0	2-Dodecylcyclobutanone (2-dDCB)
Palmitoleic Acid	C16:1	Cis-2-Dodec-5-enylcyclobutanone (2-dDeCB)

Stearic acid	C18:0	2-Tetradecylcyclobutanone (2-tDeCB)
Monounsaturated Oleic Acid	C18:1	Cis-2-tetradec-5-enylcyclo-
Di-unsaturated Linoleic Acid	C18:2	Butanone (2-tDeCB)
Linolenic Acid	C18:3	Cis, cis-2-tetradecyl-5,8-

The 2-ACBs are formed proportionally to the absorbed dose and fat content of food ³¹. The low concentration of 2-ACBs produced in irradiated foods, 0.2 to 2 mg / g fat depending on the absorbed dose, did not drive large studies On the toxicity of these molecules prior to the year 2000, since in addition to the lack of norms necessary to carry out toxicity tests it was believed that these small quantities could not promote health effects, however considering the irradiated ingredients that are added to the food and the Irradiated foods, this leads us to believe that the consumption of low concentrations of radiolytic compounds, 2-ACBs, can be acquired throughout life ³².

The formation of 2-ACBs as a function of the irradiation dose has a linear growth pattern where the rate of production is of the order of 1.0 -1.6 nmol / mmol of the precursor fatty acid, irrespective of the type of irradiation and the precursor , Which makes it possible to estimate the amount of 2-ACBs formed ³¹.

Other dietary studies have the bioavailability of 2-ACBs in adipose tissues and in a small amount in the faeces, suggesting that a smaller part is excreted and another portion is metabolized or stored in the other parts of the body ³². With the information available to date , It is premature to assert risk associated with the consumption of irradiated fat-containing products, since there are other food components that may interact with 2-ACBs, however, with evidence of toxic, genotoxic and tumor-promoting activity, more specific studies should be performed. .

Caulfield, 2008 mentions the effect of gamma irradiation on the oxidation of liposoluble vitamins in dry rations and presents the drastic reduction of its percentage after irradiation procedure, however, it does not investigate the possible products formed although this procedure is widely applied in rations for rodents and The Food and Drug Administration (FDA) has allowed as of 2001 the dose of up to 50 kGy. The present work suggests a special attention of ANVISA on the possible formation of these components in canine extruded rations, since the formation of 2-ACBs to date has not yet been studied in this type of product and in the present day this food is consumed by most dogs during Your whole life.

No adverse effects associated with the prolonged consumption of irradiated food in animals were found in the studies up to the present time. Feeding with irradiated ration for laboratory rodents is a well-established practice, however, there is little information about prolonged consumption of this type of food and Its toxicity in dogs ¹⁹. To date, there is only one study that relates the prolonged consumption of irradiated ration as a possible stimulus for the emergence of a disease in cats ³³. Despite the evolution and research, many aspects still remain unknown about the Dog nutrition, specifically on the intake of irradiated foods over months or years and the possible metabolic effects associated with their genetics and lifestyle may contribute to the manifestation of diseases ⁵.

5.CONCLUSION

Considering all the information described in the literature studies over the years and the evolution of the increasingly studied studies on the subject of 2ACBs, it was observed that there is

little information about prolonged consumption of products that have been irradiated and their toxicity in dogs. In extruded canine rations, we find in the literature mention of lipid oxidation and nutritional losses, especially of fat-soluble vitamins, however, the lack of studies on the formation of 2-ACBs and their possible toxicity, since this is normally consumed by dogs at Their lives. Since 2001, FDA / USA has accepted irradiation up to 50 kGy for microbiological control in food and in Brazil ANVISA does not suggest a specific dosage for this category of products.

The inspection and inspection of the products, destined to domestic animals, produced in Brazil or imported that are marketed in the national territory is the responsibility of the MAPA that regulates in Law nº 6,198 of December 26, 1974 the minimum quality parameters that these products must To present ³⁴, however, this law and the other Brazilian normative instructions and instructions for feeding this category of animals, until the conclusion of this work, does not mention any instruction on irradiated ingredients added to the formulation or on irradiation as a way of preserving these products. Only national quantitative parameters of nutrients to be included in feed formulation and instructions on good manufacturing practices that ensure the integrity of the final product.

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