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Original Article

Effect of Gamma Irradiation on Microbial Decontamination, Crude Nutrient Content, and Mineral Nutrient Composition of Laboratory Animal Diets

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ABSTRACT

Laboratory animal models are an important part of test design. Certain conditions such as microbial contamination in diets of these models could affect the results of experiments. One of the most important routes that predispose to contamination is generated through feeding of laboratory animals. This study aimed to show the effect of gamma irradiation in reducing bacteria concentrations, crude nutrient content, and concentrations of some minerals and trace elements in laboratory animal diets. Large-sized pellets with 10–15 mm diameter (commonly used for rats and hamsters) and small-sized pellets with 3–5 mm diameter (used for rabbits and guinea pigs) along with skimmed milk powder (SMP) as a food additive were exposed to gamma irradiation with different doses ranging from 3 to 30 kGy. The total microbial contamination and any possible changes in some mineral nutrient composition and the crude nutrient content were determined pre- and post-irradiation. Our data revealed that 25 kGy in pelleted diets and 18 kGy in SKM had superior effects in the reduction of bacterial contamination with little change in crude nutrient content and minerals and trace elements in nutrient requirements of laboratory animals. According to the results, gamma irradiation had minimal effects on crude nutrient content and the concentrations of some minerals and trace elements of laboratory animal diets, and it also eliminated bacterial and fungal contamination load. By using gamma irradiation, this method could yield a favorable outcome in controlling microbial contamination of animal diets.

Keywords: Gamma Irradiation, Laboratory animal, Diet, Bacteria, Pellet

Effet de l'irradiation Gamma sur la Décontamination Microbienne, la Teneur en Éléments Nutritifs Bruts et la Composition en Éléments Minéraux des Régimes Alimentaires des Animaux de Laboratoire

Résumé: Les modèles d'animaux de laboratoire jouent un rôle important dans la conception des tests. Certaines conditions telles que la contamination microbienne des régimes alimentaires de ces modèles pourrait affecter les résultats des expériences. L'alimentation des animaux de laboratoire est l'une des voies de contamination les plus importantes. Cette étude visait à montrer l'effet de l'irradiation gamma sur la réduction des concentrations de bactéries, teneur en éléments nutritifs et concentrations de certains minéraux et oligo-éléments dans les régimes alimentaires des animaux de laboratoire. Des granulés de grande et petite taille d'un diamètre respectif de 10–15 mm (couramment utilisés chez le rat et le hamster) et des de 3–5 mm (utilisés pour les lapins et les cobayes) contenant du lait en poudre écrémé (lait écrémé en poudre) comme additif alimentaire, ont été exposés

à une irradiation gamma à des doses allant de 3 à 30 kGy. La contamination microbienne totale et tout changement éventuel de la composition de certains éléments minéraux nutritifs et la teneur en éléments nutritifs bruts a été déterminée avant et après irradiation. Nos données ont révélé que 25 kGy en granulés et 18 kGy dans SKM ont eu des effets supérieurs dans la réduction de la contamination bactérienne avec peu de changement de la teneur en éléments nutritifs bruts, des minéraux et des oligo-éléments constituant les besoins en éléments nutritifs des animaux de laboratoire. Selon nos résultats, l'irradiation gamma a eu des effets minimes sur le contenu en nutriments bruts et sur la concentration de certains minéraux et oligo-éléments des aliments et a permis d'éliminer la charge de contamination bactérienne et fongique. En utilisant l'irradiation gamma, cette méthode pourrait aboutir à un résultat favorable dans le contrôle de la contamination microbienne des régimes alimentaires des animaux.

Mots-clés: Irradiation gamma, Animal de laboratoire, Régime alimentaire, Bactéries, Granulés

INTRODUCTION

Laboratory animals are essential components of experimental investigations, even though they are new alternative methods, annually millions of them are raised and utilized (Short, 1968). According to the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) (2017) reports about the numbers and species of animals used for scientific experimentation, about 1 million animals are used for experimentation every year (excluding rats, mice, birds, reptiles, amphibians, and agricultural animals used in agricultural experiments), plus an estimated 100 million mice and rats. Animal models play a critical role in studying human disease counterparts, testing and screening of drugs, and are indispensable parts of most in vivo experiments. In line with these comments, many efforts should be made to ensure the results of this analysis could be applicable and comparable to human medicine (Halls and Tallentire, 1978). The increasing use of germ-free and specific-pathogen-free (SPF) animals and genetically modified rodents in research have led to a growing demand for sterilized animal diets (Caulfield et al., 2008; Lee et al., 2010). In developing and producing laboratory animals, sterilization of dietary ingredients and supplements have particular importance (Hagiwara et al., 2005). Currently, different methods are used for microbial decontamination and reducing microbial load in foods; however, an approach that has minimum effect on the quality of crude nutrient content is required (DeRouchey et al., 2003; DeRouchey et al., 2004). For instance, steam sterilization methods such as autoclave change some essential ingredients of food under a high-pressure environment and/or by providing a suitable niche for the growth of microorganisms via a high moisture level. As a matter of fact, promising approaches must be designed with a low rate of side effects on food quality. Adamiker (1975) previously noted chemical sterilization and heat methods could be effectively replaced by irradiation for the reduction of bacterial and pathogen concentrations present in food (Adamiker, 1976; Al-Masri and Zarkawi, 1994). Therefore, this study aimed to determine the effects of gamma irradiation on crude nutrient content and the concentrations of some minerals and trace elements and microbial contamination by selecting optimum dose (s).

MATERIAL AND METHODS

Diet of laboratory animals. In the current experiment, two standard types of diet, small-sized pellet with 3–5 mm diameter for rabbit and guinea pig versus large-sized pellet with 10–15 mm diameter for rats and hamsters (product of Razi Vaccine and Serum Research Institute, Iran) were used for the irradiation procedure. In addition, skimmed milk powder (SMP) (product of Iran Dairy Industries Corporation) as an alternative supplement instead of milk for the cesarean

infant was selected. All pellets and additives were irradiated as described previously (Caulfield et al., 2008; Groesbeck et al., 2009). Briefly, purchased feed were emptied in separate Petri dishes – 30 g pellets (from each type) and 10 g SMP –; each Petri dishes was mixed and taped to seal it before shipment for irradiation. All separate Petri dishes were impermeable to air and irradiated using a gamma cell instrument at a dose rate of 0.65 Gy/sec. This phase was performed at Agricultural, Medical and Industrial Research School, Nuclear Science and Technology Research Institute, Karaj, Iran. The analyses were performed during 45 days in three seasons: summer, autumn, and winter. Both irradiated and non-irradiated control samples were subjected to microbial contamination and crude nutrient content besides minerals and trace elements such as calcium, phosphorus, magnesium, potassium, iron, manganese, copper, and zinc. Throughout 45 days storage, temperature and humidity were between 18–22 °C and 30–50 percent, respectively. The control diets were kept under similar temperature and humidity conditions.

Irradiation procedure. Samples from pellets and additives were subjected to gamma irradiation using a Gamma Cell Facility PX-30 system with dose rate of 0.65 Gy/sec. First, we used three different irradiation protocols as follows: in pelleted diets, the applied dose levels were exposed to 16 different doses ranging from 3 to 30 kGy; while 22 different doses were used for SMP samples, including 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, and 30 kGy. Three different sets of experiments were performed for each group. In order to determine the efficiency of irradiation on the chemical compound and microbial load, the irradiated samples with appropriate doses were packed in cellophane bags and stored for 45 days at the above-mentioned temperature and humidity.

Microbial cultures and analysis. To evaluate the rate of contaminations pre- and post-irradiation, random sampling was taken from pellets and supplements, and then isolation and enumeration of bacteria and yeasts were carried out according to previous protocols (Cowie and Makkar, 2013). MacConkey agar, tryptic soy agar, and tryptic soy broth media were used for isolation of the bacteria. Malt extract agar was used as a detection media for any S. cerevisiae contamination and sabouraud dextrose agar (SAB) was used for isolation of aspergillus. In case the bacterial or fungal colonies were observed, specific tests were used to identify the species of bacteria and fungi (Jorgensen and Ferraro, 2009). Dilutions of 10-1 were prepared for each sample and then transferred into media and incubated for 24 hours at 37 °C. In the case of no bacterial colony growth, Petri dishes were kept for another 48 hours (total 72 hours). Once too numerous to count (TNTC) colonies were achieved in the first 24 hours, further dilutions were not prepared.

Chemical analysis. Along with microbiological tests, the types and amounts of chemical compounds in pellets and supplements were determined by chemical analysis as described previously. Duplicate 30-gram bags of each treated diet (gamma-irradiated at one of the optimum doses), and duplicate 30-gram bags of each untreated diet were transported to the lab for analysis. To ensure verification of the reliability of the laboratory analyses, duplicate blinded diet samples from the same batch were analyzed and showed almost identical values. Samples (30 g) of each of these diets were analyzed for fat, protein, carbohydrate, and mineral and trace elements contents by using established analytical methodologies (Analytical-Methods-Committee, 2000; Caulfield et al., 2008). The chemical analysis was followed as: crude protein determination with the Kjeldahl method, crude fiber using the fibersics system, crude fat with Soxhlet assay, crude energy with calorimetric bombardment, and mineral elements using the atomic absorption method.

RESULTS

Determining the optimum irradiation dose for different samples. Monitoring the application of different irradiation doses showed the high-rate

microbicidal effect of 25 and 18 kGg on pelleted diets and SMP, respectively and reached to ~100% according to the data from microbial colony count analysis (Figures 1 & 2).

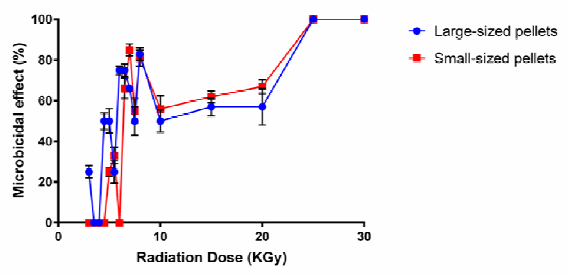

Figure 1. Determining optimum gamma irradiation dose in pelleted diets (large-sized and small-sized pellets). To calculate the percent of microbicide effects, the final upper limit of counts was estimated then the upper limit of plate counts was multiplied by the dilution factor.

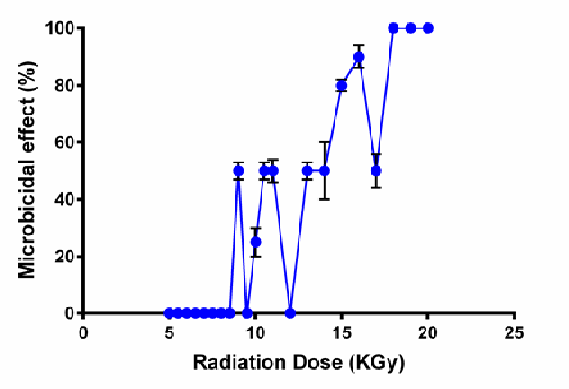

Figure 2. Determining optimum gamma irradiation dose in powdered milk.

Microbial analysis. Common bacterial contamination in pelleted diets was detected including Staphylococcus spp., Streptococcus spp., Enterobacteriaceae spp., Bacillus spp. (*B. Megaterium*, B. *Brevis*, *B. Cereus*, and *B. Coagulase*), Flavobacterium odoratum, Micrococcus varians, Pseudomonas putida, and Klebsiella pneumonia. According to data from the microbial culture, a large number of bacterial colonies were observed before the irradiation procedure. We also note the existence of fungal contamination from Aspergillus genus Aspergillus Niger and Aspergillus flavus in pellet specimens. We also confirmed the bacterial contamination in SMP from Klebsiella spp., Staphylococcus epidermis, Escherichia coli, Bacillus cereus, and Pseudomonas aeruginosa. The number of bacterial colonies was TNTC pre-irradiation, so further dilutions were prepared for sample types. Noticeably, no fungal contaminations were observed in SMP.

Chemical analysis. We performed a chemical analysis to measure the content of crude protein, fat, fiber, and energy along with mineral elements. Analyses of organic materials and mineral elements in all samples are depicted in Tables 1 and 2 pre- and post-irradiation. Based on our data, slight increases or decreases in the percentages of mineral elements and organic materials were evident after the irradiation procedure.

DISCUSSION

Laboratory animals are widely used throughout the world in various experiments in different fields of biomedicine. Unfortunately, the consequences of these experiments can be influenced by various pathogenic microorganisms, especially from food intake (Wescott and Gardner, 1962). The elimination of pathways of microorganisms to the breeding animals is a fundamental principle in housing and care of laboratory models. Up to the present, two common methods such as the application of pasteurization or gamma irradiation have been proposed for sterilization of diets (Fox et al., 2006). Food irradiation is used extensively to destroy microorganisms, a necessary process in the production of diets suitable for feeding to SPF animals. Although there is a strong demand for animal models for in vivo testing, research to determine nutrient requirements for reproduction, lactation, and maintenance of laboratory animal models has received relatively little attention. However, the nutrient requirements of rodents have been described in the reports of the Laboratory Animals Centre Diets Advisory Committee (LACDAC) (Clarke et al., 1977) and those of the National Research Council (National Research Council, Subcommittee on Laboratory

Animals, 1995). In this study, the analysis of crude fat, fiber, and protein was done following irradiation in rabbit and guinea pig diets (small-sized pellets) and rat and hamster diets (large-sized pellets) along with SMP samples. Data demonstrated only crude protein in large-sized pellets, and crude fat in SMP did not change, but the rest of the components showed slight changes. To understand how the nutritional content of these diets was changed following irradiation and compare the values with standard protocols, it is thought necessary to refer to NRC recommendations and protocols (National Research Council, Subcommittee on Laboratory Animal, 1995). According to the NRC nutrient requirements recommendation for maintenance, growth, and reproduction of laboratory animals (4th revised edition 1995), the amounts of protein for growth of laboratory rats, mice, and guiana pigs are estimated at 150, 180, 180 g per kg diet, respectively. It is thought that fat requirement reaches 50, 50, and 4 (essential fatty acids), respectively. Nutrient requirements for gerbils are the same as recorded for rats. Nutrient requirements of rabbits based on NRC's Nutrient Requirements of Rabbits (1977) is: crude fiber (10–12 %), crude fat (2%), and crude protein (16%). These levels are different for maintenance, gestation, and lactation purposes. Based on the results from the current experiment, the values before and after irradiation are comparable to dietary standards for laboratory animals. According to these recommendations, it is well known that gamma-irradiation has only a small effect (if at all) on the macronutrients like crude protein, but for unknown

Table 1. Analysis of organic material in pelleted diets and skimmed milk powder (SMP) pre- and post-irradiation. Thirty-gram pellet specimens were subjected to chemical analysis before and after gamma irradiation with optimum doses (25 kGy and 18 kGy for pellets and SMP, respectively). The values are expressed as percentage of pre-irradiation levels.

Sample	Percent of organic material (average)			
	Crude protein (%)	Crude fiber (%)	Crude fat (%)	Crude energy (kCal/Kg food)
Non-irradiated large-sized pellets (control)	18.4	9.6	4.35	4296.2
Irradiated large-sized pellets	18.6 (101%)	8.4 (87.5%)	4.05 (93.1%)	4303.5 (100.1%)
Non-irradiated small-sized pellets (control)	12.9	12.6	2.5	4301.8
Irradiated small-sized pellets	14.4 (111.6%)	11.6 (92%)	2.02 (80.8%)	4294.9 (99.8%)
Non-irradiated SMP (control)	28.6	_	0.2	4094.6
Irradiated SMP	32.4 (113.2%)	_	0.2 (100%)	4088.3 (99.84%)

Table 1. Mineral elements were measured in pellets and SMP samples before and after-irradiation. Pellets and SMP were exposed to 25 kGy and 18 kGy of gamma irradiation, respectively. The values in parenthesis are expressed as the percentage change from pre-irradiation levels.

Sample	Mineral elements levels							
	Calcium (%)	Phosphorus (%)	Magnesium (%)	Potassium (%)	Iron (mg/kg)	Manganese (mg/kg)	Copper (mg/kg)	Zinc (mg/kg)
Non-irradiated large-sized pellets (control)	1.38	0.83	0.39	0.91	39.40	43.1	16.6	18.7
Irradiated large-sized pellets	1.73 (125%)	0.89 (107%)	0.36 (92.3%)	0.93 (102%)	33.86 (85.9%)	35.7 (82.8%)	14.8 (89.1%)	18.15 (97%)
Non-irradiated Small-sized (control)	1.40	0.64	0.61	1.89	12.11	70.8	24.2	15.7
Irradiated small-sized pellets	1.53 (109%)	0.65 (101%)	0.37 (60.6%)	1.1 (58.2%)	11.98 (98.9%)	37.9 (53.5%)	13 (53.7%)	11.2 (71.3%)
Non-irradiated SMP **(control)**	1.28	1.08	0.42	1.38	4037	24.6	5.3	21.1
Irradiated SMP	1.81 (140)	1.40 (129%)	0.31 (73.8%)	1.85 (134%)	3901 (96%)	21.2 (86.1%)	6.2 (116.9%)	15.2 (72%)

reasons, our results are slightly different. Since the current experiment had been designed to investigate the effect of gamma irradiation on food requirements of laboratory animals, the observed results may not be comparable to work done by Caulfield et al. In contrast to our results, they observed some changes after irradiation or pasteurization on animal diets (Caulfield et al., 2008). However, the doses used in our study were similar to doses applied by Caulfield et al. (2008). Based on their reposts, levels of some vitamins and nutritional components in dry rodent food before and after gamma irradiation or pasteurization changed, and consequently, they have recommended that irradiated diets of rodents should be used with caution during long-term exclusive feeding of cats. According to the findings of the current study, some level changes in minimal daily intake values of each nutrient was shown for the growth and reproduction of laboratory animals. Considering being an alternative option in decontaminating of animal diets, we also suggest deciphering the potent possible harmful effects of irradiated foods in long-term use in producing laboratory animals, especially in SPF animals. Commensurate with these descriptions, data from our experiment and previous studies confirmed the efficiency of gamma irradiation in preserving crude nutrient content approved by NRC guidelines. Next, we went on to analyze the effect of gamma irradiation on mineral elements in pelleted diets and SMP. The results showed that only phosphorus levels did not change after irradiation. No obvious changes were found for other minerals. According to given data, the amounts of bacterial contamination in pelleted diets (samples that have only bacterial contamination) and SMP were TNTC pre-irradiation. It is worth noting that bacterial colonies in the large and small-sized pellets were more than fungal contamination. Calling attention, the optimum doses for eliminating these contaminations were 25 and 18 kGy in pelleted diets and SMP, respectively. These values are similar to commercially adopted gamma sterilization doses for SPF diets (Furuta et al., 2002). Irradiation doses ranging from 20 to 30 kGy are currently used to treat diets intended for SPF animals (Fox et al., 2006). Difference between optimum doses of SMP and pelleted diets possibly suggests the number of resistant bacteria to irradiation. Although autoclaving and dry heat are inexpensive and common methods of sterilization, the entity of diet ingredients may be strongly influenced by humidity and heat (DeRouchey et al., 2003; DeRouchey et al., 2004). In chemical sterilization such as ethylene oxide, the production of toxic compounds is almost inevitable. With the emergence of food packaging technology and food irradiation, it is reasonable to think that gamma-irradiation induced minor alterations are not detrimental to food ingredients and consequently fully meet the requirements for animal's diets (Morehouse and Komolprasert, 2004). As mentioned above, irradiation of laboratory animal diets in the form of SMP and pelleted diets using gamma irradiation is a safe and secure procedure (DeRouchey et al., 2003; Arvanitoyannis and Stratakos, 2010; Simas et al., 2010). Not only this method has extreme impacts on food by eliminating bacterial and fungal contamination load, but also provides the possibility of food packaging in custom sizes and shapes. It is pointed out that utilized dose(s) on diets are safe in laboratory animals (Chiang et al., 2010). Gamma irradiation has possibly, however, an effect on fatty acids (FA), particularly on unsaturated FA, some amino acids, and vitamins. Thus, it would have been more meaningful to analyze the concentration of individual amino acids, activity of some vitamins, and potential oxidation products, and probably the fatty acid profile.

Overall, gamma irradiation as a novel approach in sterilizing food requirements yields a favorable outcome in controlling microbial contamination of animal diets.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgment

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