VARIATION IN SENSITIVITY OF SELECTED BACTERIAL PATHOGENS FOR GAMMA RADIATION

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Abstract

Bacteriological examination of 100 samples of animal feed stuff and 50 samples of pet feeds revealed the isolation of different bacterial pathogens mainly: *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Proteus* spp., *Staphylococcus aureus* and Salmonella spp. in a decreasing order of occurrence. The efficacy of Gamma irradiation against the inoculated bacterial isolates (*E. coli* O157: H7, *S. enteritidis* and *S. aureus*) in feed stuff and pet feeds was investigated. Irradiated samples were stored at room temperature (25ºC) for 2 weeks.

The feed borne pathogens used in this study showed difference in radiation sensitivity. *E. coli* O157: H7, *S.* enteritidis and *S. aureus* were sensitive to ionizing radiation at 1 KGy, 3 KGy and 1/2 KGy, respectively. Also, inoculated pathogens in semi moist pet feeds were more sensitive to ionizing radiation than dry pet feeds.

The irradiation sensitivity of bacterial isolates under planktonic or biofilm conditions were determined. Selected isolates were grown in tryptic soya broth, and biofilms were allowed to form on sterile glass slides. Both biofilm and the broth cultures were irradiated at doses of 0.0 (control), 0.5, 1, 2, 3 and 4 KGy. The results revealed that *E. coli* O157: H7 and *S. enteritidis* for biofilm associated cells were more sensitive to ionizing radiation than the respective planktonic cells, while, for *S. aureus*, the reverse was observed. It was concluded that irradiation is an effective means of inactivating pathogenic bacteria. This radiation sensitivity is related to the bacterial isolates and the evaluated growth form (Planktonic versus biofilm associated cells). Despite the variation in radiation sensitivity of planktonic and biofilm associated pathogens, the antimicrobial efficacy of the process indicated that irradiation is an effective means of inactivating bacteria, even within biofilm.

INTRODUCTION

Escherichia coli O157: H7, *Salmonella* spp. and *S. aureus* are important feed borne pathogens responsible for feed borne illness associated with a variety of feed products (Rajkowski and Thayer, 2000).

Escherichia coli O157: H7 was the main serotype implicated in large outbreaks of haemorrhagic colitis and haemolytic uraemic syndrome (Besser *et al.*, 1997), also, may produce diarrhoea in human and animals (Wilson *et al.*, 1996). FDA (2001) suggested that pets with Salmonella infection may be lethargic and have diarrhoea or bloody diarrhoea, fever, and vomiting. Some pets may have only decreased appetite,

fever and abdominal pain, while, apparently healthy animals can be carriers and infect other animals or human.

Animal feed stuffs and feed of pet animals may play a role in the transmission of these pathogens which are of zoonotic importance, moreover, numerous studies have documented the ability of *E. coli* O157: H7, *Salmonella* spp. and *S. aureus* to adhere and form biofilm on different surfaces such as plastic, cement, glass and stainless steel (Guillot *et al.*, 2007).

Biofilm has been defined as a community of bacteria living in organized structures at a liquid interface (Davies, 2003). Microscopic investigations of biofilm structure have revealed that bacteria exist in microcolonies that are encapsulated in a matrix of extra cellular polymeric material (Starkey *et al.*, 2004). The presence of these organisms in feed processing environments can serve as a persistent source of contamination.

Biofilm associated bacteria have been demonstrated to be more resistant to antimicrobial treatments compared to their planktonic counter parts (Niemira and Solomon, 2005).

Irradiation process which causes chemical changes, is already approved for use on a variety of human foods. Extending this process to animal feed and feed ingredients including pet feeds will increase the safety of the feed for both the animals consuming it and the people handling it (Niemira, 2007).

The aim of this work was firstly directed to determine the occurrence of bacterial pathogens from animal feed and pet feeds, secondly to determine the variation in sensitivity of inoculated feed borne pathogens (*E. coli* O157: H7, *S. enteritidis* and *S. aureus*) in animal feedstuff and pet feeds for Gamma irradiation, and also, to investigate the relative susceptibilities of planktonic cells versus those of biofilm associated cells of the isolated selected bacterial feed borne pathogens to Gamma irradiation.

MATERIALS AND METHODS

Bacteriological examination of animal feeds and pet feeds was carried out according to Collins and Cummins (1986). A total of 100 samples of animal feed stuffs and 50 samples of pet feeds were obtained from serology unit, Animal Health Research Institute, Dokki, Giza. These samples consisted of 50 ones from each of fish meal and bone and meat meal, while, from pet feeds semi-moist and dry pellets, 25 samples from each were collected.

Out of each sample, 25 g were inoculated into 250 ml peptone water (enrichment broth), incubated at 37°C for 24 h and then, cultivated onto- blood agar,

nutrient agar and MacConkey agar media and incubated for 24 h at 37°C. Also, 1 ml of each, the inoculated peptone water was transferred to 9 ml selenit "F" broth (pre-enrichment fluid media, Oxoid) and incubated at 37 C° for 12-18 h. An inoculum was then cultured onto *Salmonella - Shigella (S.S.)* agar medium. After over night incubation of MacConkey agar, a part of single typical well isolated lactose fermenting colonies was tested for sorbitol fermentation by cultivating onto sorbitol MacConkey agar (S.M.A.) (Oxoid), and incubated at 37°C overnight. From sorbitol MacConkey agar, colourless or pale colonies were considered as non-fermenters of sorbitol and pink colonies as sorbitol fermenters. Non-sorbitol fermenter colonies on S.M.A. and non-lactose fermenter colonies with or without black center on S-S media were examined culturally, morphologically, biochemically, as well as serologically. Suspected colonies of *S. aureus* from blood agar and nutrient agar were confirmed by coagulase, catalase and anaerobic utilization of glucose and mannitol.

Serotyping of *E. coli* O157:H 7 was applied on non sorbitol fermenting isolates using *E. coli* antisera polyvalent 3 and monovalent antisera O157, and H7. Antisera were obtained from Denka Seiken Laboratory, Tokyo. Japan.

Serotyping identification of isolated strains of salmonellae was performed according to "Kauffman white scheme" using slide agglutination test for identification of somatic antigen, while, flagellar antigen was identified using tube agglutination test. The antisera were obtained from Bio-Rad laboratories, France.

Variation in sensitivity of bacterial isolates for Gamma radiation was employed according to Khattak *et al.* (2005).

1. Preparation of inocula

Pure cultures of *E. coli* O157: H7, *S. enteritidis* and *S. aureus* previously isolated from animal feed stuffs were used. Each bacterial isolate was grown individually in tryptic soya broth (T.S.B., Difco) for 18 h to a concentration of 8 log cfu / ml. The cultures were diluted and suspended in sterile 0.1% peptone water.

2. Radiation process

Twenty-five g of each sample (fresh meal, bone and meat meal, semi-moist and dry pet feeds) were packed in polyethylene packages and sterilised in autoclave at 120°C under 1.5 P. for 20 minutes. After that, each sample was inoculated with pure culture of one tested pathogen previously prepared (*E. coli* O157: H7, *S. enteritidis* and *S. aureus*). The polyethylene bags were then sealed and marked with specific doses of irradiation. The irradiation process was carried out using the Cobalt 60 Egypt Gamma-1 irradiator, located at the National Center for Radiation Research and Technology (N.C.R.R.T) Nasr city, Cairo, Egypt.

The samples were irradiated at doses of 0, 0.5, 1, 2, 3, 4 and 5 KGy with a gamma source. The irradiated and control samples (triplicate) were kept at room temperature for bacteriological examination at 0, 7 and 14 days.

3. Bacteriological examination

Five grams of each sample were placed in 45 ml of sterilized diluent (0.1% peptone water) and thoroughly mixed according to Collins and Lyne (1976). Serial dilutions were made and 1 ml of each dilution was plated on tryptic soy agar (T.S.A). Two Petri plates were used for each dilution. Plates were incubated at 37°C for 24 to 48 h and the colonies were counted, the bacteriological examination was applied at 0, 7 & 14 days.

Variations in sensitivity of planktonic and biofilm associated cells for gamma radiation

1- Preparation of planktonic and biofilm associated cells was done according to Niemira and Solomon (2005) for each bacterial isolate, sterile TSB with 200 µl of a stock culture was incubated overnight at 37°C to make a fresh culture (approximately 10^9 cfu/ ml). Sterile glass microscope slides were aseptically placed into 50 milliliters tubes containing 25 ml of sterile TSB and inoculated with 200 µl of the fresh culture. Tubes were hold upright in a rack and incubated at 37 C° for 72 h. This configuration resulted in proximally 3.5 cm of the slide being submerged in culture medium, the upper part of the slide in the headspace of the tube remained dry. The test was performed in duplicate.

2. Microscopic observations

Microscopic observations of adherent cells were made after staining the cells with crystal violet stain.

3. Irradiation

Planktonic cell and biofilms were irradiated using gamma radiation doses of 0.5, 1 and 2 for *E. coli* O157: H7 and 2, 3 and 4 for *S. enteritidis*, while, with *S. aureus* the doses were 0.5, 1 and 2 as mentioned by Niemira and Solomon (2005) and Niemira (2007).

4. Enumeration of survivors

Tubes were opened, and surviving planktonic cells were enumerated by withdrawal of 1 ml aliquot of the liquid culture (extreme care was undertaken to avoid contacting either the glass slide or the sides of the culture tube). Serial dilutions with phosphate buffer saline and pour plating using T.S.A biofilm – associated cells were enumerated by removing the microscopic slide using sterile forceps to grip the clean, dry upper portion of the slide, rising for two seconds under a stream of sterile distilled water to remove unattached cells, and then, shaking the slide in 25 ml of PBS in a sterile 50 ml centrifuge tube. Aliquots were then plated using TSA and incubated overnight at 37°C.

RESULTS

Results of bacteriological examination of animal feed stuffs and pet feeds are illustrated in Table 1 showing that, 6 different types of bacterial pathogens were recovered. The most prevalent isolated bacteria were *E. coli* 56 (37.3%), 11 (7.3%), from which, there were non-sorbitol fermenting, *Klebsiella* spp. 29 (19.3%), *P. aeruginosa* 28 (18.7%), *Proteus* spp. 26 (17.3%), *S. aureus* 19 (12.7%), while, *Salmonella* spp. 4 (2.6%) were isolated only from bone and meat meal.

Out of 11 (7.3%) *E. coli* non-sorbitol fermenting colonies 5 (3.3%) were *E. coli* O157: H7.

Serological identification of 4 isolates of *Salmonella* spp. revealed that they belonged to 4 different serovars including *S. enteritidis*, *S. virshow*, *S. senftenbery* and *S. montovideo*.

Table 1. Bacteriological examination of animal feed stuffs and pet animal feed.

Bacterial isolates	Fish meal N =50	Bone and meat meal m =50	Pet feed		Total m=150
			Dry pellets m=25	Semi moist m=25	
E. coli	20 (40%)	22 (44%)	8 (32%)	6 (12%)	56 (37.3%)
E. coli O157	1 (2%)	6 (12%)	3 (12%)	1 (4%)	11 (7.3%)
E. coli O157:H7	0 (0%)	3 (6%)	2 (8%)	0 (0%)	5 (3.3%)
Salmonella spp.	0 (0%)	4 (8%)	0	0	4 (2.6%)
Proteus spp.	11 (22%)	9 (18%)	4 (16%)	2 (8%)	26 (17.3%)
Klebsiella spp.	10 (20%)	8 (16%)	6 (24%)	5 (20%)	29 (19.3%)
Pseudomonas aeruginosa	13 (26%)	10 (20%)	4 (16%)	1 (4%)	28 (18.7%)
S. aureus	5 (10%)	6 (12%)	3 (12%)	5 (20%)	19 (12.7%)

Percentage was calculated in relation to No of each type of examined sample.

Bacteriological changes in control and irradiated feed stuffs

1. *E. coli* O157: H7 inoculated samples

In control samples, bacterial counts increased from a range of 10^4 to 10^6 of cfu/g after 14 days of storage (Table 2). For irradiated dry pet feed samples, the count ranged from 1×10^3 (0.5 KGy) to 1×10^2 (1 KGy) cfu/ g on 0 day of storage. Bacterial counts changed slightly after 7 days of storage to 2×10^3 and 2×10^2 cfu/ g for samples irradiated at 0.5 and 1 KGy, respectively. These values were further increased on 14 days of storage to 4×10^3 and 3×10^2 cfu/ g for 0.5 and 1 KGy. At 2 KGy irradiated samples no growth of O157: H7 in dry pet animal feed was detected.

The remaining samples (fish meal, bone and meat meal and semi moist pet animal feed), the bacterial count decreased with increasing irradiation dose. At 1 KGy irradiated, samples showed no growth of *E. coli* O157: H7.

2. *S. enteritidis* inoculated samples

The control animal feed and pet feed samples had bacterial count ranging from 1×10^4 to 4×10^5 cfu/ g after 14 days of storage. The 3 KGy irradiated samples had no bacterial count after the same storage periods except dry pet animals feed at 3 KGy irradiated samples in which the count was less than 100 cfu/ g on 0 day, whereas, at 4 KGy irradiated samples showed no growth of *S. enteritidis.*

3. *S. aureus* inoculated samples

S. aureus counts also showed a similar trend. The 0.5 KGy irradiation showed that all samples had no count after 14 days of storage except dry pet animal feed samples which had a *S. aureus* count of 10^2 cfu/ g that increased at 3×10^3 after 14 days of storage.

Table 2. Bacteriological changes in control and irradiated animal feed stuffs.

Inoculated bacterial pathogen	Animal feed stuffs	Radiation dose	Storage period day		
			0	7	14
E. coli O157:H7	Fish meal	0 (control)	10^4	2×10^4	1×10^5
		½	10^3	1×10^2	2×10^3
		1	No growth		
		2			
		3			
		4			
		5			
	Bone and meat meal	0	10^4	3×10^4	1×10^5
		½	2×10^4	3×10^4	1×10^5
		1	No growth		
		2			
		3			
		4			
		5			
	Semi moist pet feed	0	10^5	2×10^5	10^6
		½	10^3	10^4	10^4
		1	No growth		
		2			
		3			
		4			
		5			
	Dry pet feed	0	10^4	2×10^4	1×10^5
		½	10^3	2×10^3	4×10^3
		1	10^2	2×10^2	3×10^2
		2	No growth		
		3			
		4			
		5			

Table 2. ... continued

Inoculated bacterial pathogen	Animal feed stuffs	Radiation dose	Storage period day		
			0	7	14
S. enteritidis	Fish meal	0 (control)	1×10^4	2×10^4	5×10^4
		½	2×10^2	2×10^2	3×10^2
		1	10^2	1×10^2	2×10^2
		2	Less than 100	10^2	2×10^2
		3	No growth		
		4			
		5			
	Bone and meat meal	0	2×10^4	3×10^4	3×10^5
		½	1×10^4	2×10^4	3×10^4
		1	10^3	2×10^3	2×10^3
		2	1×10^2	2×10^2	3×10^2
		3	No growth		
		4			
		5			
	Semi moist pet feed	0	2×10^4	3×10^4	4×10^5
		½	1×10^3	2×10^4	3×10^4
		1	1×10^3	2×10^3	3×10^3
		2	2×10^2	3×10^2	4×10
		3	No growth		
		4			
		5			
	Dry pet feed	0	2×10^4	3×10^5	4×10^4
		½	1×10^2	2×10^4	2×10^4
		1	10^4	2×10^3	3×10^3
		2	1×10^2	1×10^3	1×10^3
		3	Less than 100	1×10^2	2×10^2
		4	No growth		
		5			

Table 2. ... continued

Inoculated bacterial pathogen	Animal feed stuffs	Radiation dose	Storage period day		
			0	7	14
S aureus	Fish meal	0 (control)	1×10^4	2×10^5	5×10^5
		½	No growth		
		1			
		2			
		3			
		4			
		5			
	Bone and meat meal	0	1×10^4	3×10^4	4×10^4
		½	No growth		
		1			
		2			
		3			
		4			
		5			
	Semi moist pet feed	0	1×10^4	2×10^4	1×10^5
		½	No growth		
		1			
		2			
		3			
		4			
		5			
	Dry pet feed	0	2×10^4	4×10^4	3×10^5
		½	1×10^2	2×10^2	3×10^3
		1	No growth		
		2			
		3			
		4			
		5			

Variation in sensitivity of planktonic and biofilm associated cells for gamma radiation

For *E. coli* O157: H7 and *S. enteritidis*, the planktonic cells had radiation dose higher than that of the biofilm associated cells. For *S. aureus*, the reverse was observed with radiation dose of the biofilm associated cells being higher than that of the planktonic cells.

Table 3. Variation in sensitivity of planktonic and biofilm associated cells for gamma radiation.

Bacterial isolates	Culture	Radiation dose	cfu/ ml
E. coli O157: H7	Planktonic	0 control	10^6
		0.5	10^2
		1	No growth
		2	No growth
	Biofilm	0 control	10^5
		0.5	No growth
		1	No growth
		2	No growth
S. enteritidis	Planktonic	0 control	10^6
		2	10^3
		3	No growth
		4	No growth
	Biofilm	0 control	10^4
		2	No growth
		3	No growth
		4	No growth
S. aureus	Planktonic	0 control	10^5
		1/2	No growth
		1	No growth
	Biofilm	0 control	10^4
		1/2	10^2
		1	No growth

DISCUSSION

Animal feeds and feed of pet animals are frequently contaminated with feed borne pathogens, which may cause infection in animals leading to subsequent contamination of carcasses and human food–borne infections.

Data presented in Table 1 show the variation in bacterial isolates from different kinds of animal feed stuffs and pet feeds. The occurrence of bacterial isolates in decreasing order were *E. coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *proteus* spp., *S. aureus* and *Salmonella* spp.

These results are nearly in agreement with those reported by Hacking *et al.* (1978) who found *Salmonella* in 3% of the pelleted feed samples from one commercial mill in Canada, and concluded that pelleting failed to eliminate *salmonellae.* Cox *et al.* (1983) isolated Enterobacteriaceae in 100, 60 and 92% and *Salmonella* from 58, 0 and 92% of mash, pelleted, and meat and bone meal samples. Although no *Salmonella spp.* were found in the pelleted samples, the presence of other Enterobacteriaceae suggests that commercial pelleting may not totally destroy *Salmonella* spp. since their heat resistance is similar to other founded organisms. Also, McCapes *et al.* (1989) recovered *Escherichia coli* from 72-100% of the samples of raw mash, and *Salmonellae* (*S. senftenberg*, *S. bredeney* and *S. mbandaka*) were isolated from 5 to 10% of samples of raw mash.

These results indicated the need for the enforcement of preventive and control measures for reducing the public health and animal health hazards of feed borne pathogens.

Irradiation effectively eliminates spoilage and pathogenic microorganisms in food, the treatment of food by ionizing radiation is a technological process which enhances the hygienic quality of food, and contributes to the reduction in the incidence of illness caused by food borne pathogens.

As shown in Table 2, *E. coli* O_{157}: H_7, *S. enteritdis* and *S. aureus* were sensitive to ionizing radiation at a dose of 1 KGy, 3 KGy and 1/2 KGy, respectively. These results agreed with those reported by Gibbs and Wilkinson (1985) who found that Gram- negative rod shaped bacteria belonging to Enterobacteriaceae group and pseudomonas are among the most radiation sensitive microorganisms. Prakash *et al.* (2000) found that 1 KGy treatment eliminated inoculated *E. coli.*

Also, Table 2 revealed that dry pet feeds are resistant to irradiation than semi moist pet feeds. These results could be explained by Niemira (2003), who suggested that irradiation acts via radical molecules created when high – energy particles split water molecules within the product or suspending environment and within the resident bacteria. These radicals damage cellular and biochemical structures that they come in contact with, such as nucleic acid strands, cell membranes and protein structures. Under conditions of limited free water such as dry or frozen products, the mobility of these radicals is limited increasing the likelihood of the radicals self quenching rather than damaging the nearly bacterial cells. In these protective environments, higher

doses of irradiation are required to affect equivalent log reductions of target pathogens resulting in high radiation doses.

From these results, it was concluded that ionizing radiation effectively reduced the population of bacterial isolates. This reduction was dose related. The mechanisms responsible for bacterial species specific differences in sensitivity to ionizing radiation are complex and have not yet been fully elucidated.

The three bacterial food borne pathogens produced biofilm under the condition utilized in our experiments. Results in Table 3 revealed differences in the radiation resistances related to the growth form evaluated (planktonic versus biofilm associated cells). In case of *E. coli* O157: H7 and *S. enteritidis*, the planktonic cells were more resistant to radiation than biofilm associated cell, while, with *S. aureus*, the reverse occurred (Table 3).

These results agreed with those obtained by Niemira and Solomon (2005) who suggested that biofilm associated *Salmonella* cells were recently shown to be either similarly susceptible or more susceptible to ionizing radiation than the corresponding planktonic cells.

Also, Niemira (2007) found that ionizing radiation effectively inactivates *E. coli* O157: H7, but, the efficacy of the process against biofilm cells versus that against free- living planktonic cells is not well documented. On the other hand, Sommers (2003) suggested that the primary mode of action of irradiation is via oxygen and hydroxyl radicals, solutions with high antioxidant capacity are known to protect suspended bacteria by neutralizing these radicals before they can damage bacterial cell membranes or other structures, there by reducing the efficacy of the process in this aspect. Niemira (2003) suggested that biofilm expolysaccharides (EPS) microstrctural elements have been proposed as a mechanism by which biofilm associated pathogens either might be protected from irradiation or might be a source of hydroxylradicals, thereby enhancing the antimicrobial effect.

In general, irradiation of animal feed stuff and pet animal feed will increase the safety of the feed for both animals consuming it, and the people handling it. Despite the variation in radiation sensitivity, the antimicrobial efficacy of the process against biofilm associated pathogens indicates that irradiation remains as an effective means of inactivating bacteria, even within a biofilm. This radiation sensitivity is related to the bacterial isolates and the evaluated growth from (planktonic versus biofilm associated cells).

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التنوع فى حساسية البكتريا الممرضة المختارة لأشعة جاما

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٢. *المركز القومى لبحوث وتكنولوجيا الإشعاع – مدينة نصر – القاهرة*

تم إجراء الفحص البكتريولوجى لعدد ١٠٠ عينة من أعلاف الحيوانات و ٥٠ عينة من أغذية الحيوانات الأليفة وأظهرت النتائج عزل ميكروب الايشريشيا كولى (الميكروب القولونى) وميكروب الكلبسيلا والميكروب الصديدى الأخضر وميكروب البروتيس والميكروب العنقودى الذهبى وميكروب السالمونيلا ترتيباً تنازلياً تبعاً لنسبة تواجدها. وقد تم دراسة كفاءة أشعة جاما على ثلاثة من المعزولات وهى ميكروب الايشريشيا كولى O157:H7 ، وميكروب السالمونيلا انترتيدس والميكروب العنقودى الذهبى وتم العد البكتيرى لمدة أسبوعين.

وقد وجد أن حساسية الميكروبات المستخدمة مختلفة لأشعة جاما وكان ميكروب الايشريشيا كولى حساساً لأشعة جاما عند تعرضه عند 1 KGy بينما ميكروب السالمونيلا انترتيدس عند 3 KGy والميكروب العنقودى الذهبى عند ½ KGy. كما أثبتت النتائج أن الميكروبات المحقونة فى أغذية الحيوانات الأليفة شبه الرطبة أكثر حساسية من المحقونة فى أغذية الحيوانات الأليفة الجافة.

وقد تم دراسة حساسية الميكروبات المعزولة المختارة المنزرعة فى مستنبتات سائلة والآخرى المكونة للغشاء الحيوى. الميكروبات المختارة نمت على شربة الصويا. والغشاء الحيوى تكون على الشرائح الزجاجية وتم تعريض كل من النوعين للإشعاع عند جرعات (٠) جرعة ضابطة، ٠,٥ ، ١، ٢، ٣، ٤ KGy وكانت النتائج كالآتى: الغشاء الحيوى لميكروب الايشريشيا كولى O157:H7 ، وميكروب السالمونيلا كل منها أكثر حساسية من الموجودة فى سائل لكل منها، والعكس صحيح مع الميكروب العنقودى الذهبى. وتوصى النتائج بكفاءة الأشعاع للحد من التلوث البكتيرى وأن الأشعاع ذو كفاءة سواء فى الحد من التلوث بالبكتيريا أوالقضاء على أنواع البكتيريا المكونة للغشاء الحيوى بالرغم من أختلاف حساسية الميكروبات المكونة للغشاء الحيوى والأخرى الموجودة فى سائل. وقد أثبت البحث كفاءة أشعة جاما فى الحد من تلوث الأعلاف وأغذية الحيوانات الأليفة وكفاءتها فى الحد من التلوث بالبكتيريا المكونة للأغشية الحيوية.