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Impact of Irradiated Soya mix on Growth and Development of Weanling Albino rats

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Abstract: **Food is pre-requisite for food security and it provides economic stability and self-reliance to a nation. Macro and micronutrient malnutrition is more prevalent in developing and underdeveloped countries because of food insecurity. Food security is a major challenge that is encountered by many under developed, developing and developed nations of the world today. Several advanced preservation techniques have been developed for extending the shelf life of food commodities. Food irradiation is one such modern processing technology describing as a versatile, efficacious and safe process. In this direction the present study is an attempt to study the impact of radiation processing of formulated soya mix. The low dose levels 0.25 kGy and 0.75 kGy was used for radiation processing to retain the nutrient quality of the food and to maintain the safety and stability. The prepared and processed health mix was supplemented to weanling rats to compare the nutritional quality of irradiated and non-irradiated soya mix through animal experimentation. The ten days old Wister albino rats were selected for experiment, the feeding was given for 5 weeks. After the termination the liver weight, bone weight and length, bone calcification, liver and faecal nitrogen and hemoglobin % was estimated in albino rats. The liver nitrogen, faecal nitrogen and haemoglobin percentage was high in irradiated samples than the non- irradiated samples. The difference between the irradiated and non-irradiated samples was found to be significant @ 1% level.**

Keywords: **Food security, Soya mix, Food processing, Radiation processing.**

1. INTRODUCTION

Soy beans are considered as a healthy food and compositionally it has high protein, low fat, phytochemicals of biological significance. The soy protein has a high biological value and contains all essential amino acids (Liu, 2012). Food mixes are developed with the purpose to provide nutritionally high biological value protein and also to use as concentrated sources of energy, iron, folic acid and other specific nutrients particularly the micronutrients. The health benefits of soybeans are recognized for millennia.

Food safety appears to be one of those important public health issues which is only recently gaining the required momentum. In promoting food supply and proper nutrition at least two factors have to be considered. There is first of all, the availability of food in sufficient amounts and of the right nutritional content in relation to such factors as age, occupation and climate, secondly and equally important, this food has to safe, which implies that its consumption should not give rise to food borne disease, whether from infection or intoxication (Fanzo, 2015). Consumer standard for food safety has been increasing worldwide. Thus the development of processing procedures for safe and wholesome foods or public health related products are required to meet the consumer's standards. Food irradiation technology is widely adopted because of its effectiveness technically and economically.

Food irradiation has gone through a number of phases, beginning with research and development in the 1960's going on to evaluation of safety and wholesomeness in the 1970's and to the establishment of national and international standards in the 1980's. 35 countries already have regulations allowing irradiation for processing of one or more food items and the list is growing (Komolprasert et al., 2004).

Laboratory animals themselves act as measuring instruments for toxicological investigations. It is vital to investigate the effect of feed- sterilization processes on the well being of the laboratory animals (Conning, 1967). Many research studies were carried out on food irradiation with animal experimentation generally a group of animals, mainly rats were fed a diet consisting of the food under trail (irradiation food) and their growth, longitivity, metabolic activity etc., were compared with those obtained with the non – irradiated food. Extensive studies were carried out and the parameters studied were body weight, hematological studies, clinical blood chemistry, urine analysis, organ weight, histopathology, post mortem observations, data on fertility, litter size, birth weight, weaning weight, lactation etc.

Irradiation is increasingly used as a method for routine sterilization of laboratory animal feed and there is now considerable experience of the application of δ-irradiation and much research into the comparative effects of the alternative methods of sterilization on the wholesomeness of the diet. Low dose radiation process is now widely recognized as an effective method for extending the shelf life of cereals and legumes. In this context, health mixes were selected for the current study to know the effect of radiation processing of the health mix on quality, shelf life and acceptability of the irradiated soy mix.

2. METHODOLOGY

Food mixes are developed with the purpose to provide nutritionally high biological value protein and also to use as concentrated sources of energy, iron, folic acid and other specific nutrients particularly the micronutrients. The health benefits of soybean are recognized for millennia. In recognition of the many advantages of soy flour as a protein supplement, considerable effort has gone into blending soy flour with cereals. In addition to their significant levels of high quality protein, when suitably fortified with vitamins and minerals, have great potential for feeding people of all age groups.

Formulation of Soy Mix

The formulation of soya mix was done with the combination of cereals (45%), pulses (50%). Cereals used were parboiled rice, rice flakes and jowar; pulses were mainly soya bean and roasted bengal gram and 5 % of sugar.

Method of Preparation

All the ingredients were cleaned and weighed, soya beans were soaked overnight and grains were washed and dried in sunlight until it is dried. Rest of the ingredients, jowar, rice flakes and parboiled rice were cleaned and roasted lightly. All the ingredients were ground into fine powder with sugar.

Packaging and Labeling

The product was packed in High Density Poly Ethylene (HDPE) packs. The packs were in two sizes of 250 g and 50 g. 250 g packet was for feeding the animals and 50g packets were sample packs used for shelf life studies. Each pack was sealed and labeled. The details on the label include date of manufacture, radiation status, level of radiation and its intended use.

Radiation Processing of Health Mix

After packaging and labeling the health mix was stored at room temperature till the time of irradiation. In the present study, low dose levels 0.25 kGy and 0.75 kGy employed for irradiation for 50g and 250g of Soya health mix. The irradiation was done in gamma chamber in irradiation plant at Food irradiation unit at Quality control lab, Prof. Jayashankar Telangana state Agriculture University, Hyderabad.

The irradiated health mix samples were stored at both ambient and refrigerated temperatures. After 15 days of irradiation the mixes were used for different tests including animal experiment.

Selection of the albino rats and their distribution into different treatments

Ten days old weanling albino rats were purchased from the NIN (National Institute of Nutrition), Hyderabad. The rats were fed two to three days with NIN standard diet till the experiment begins. There were 6 animals, 3 males and 3 females per treatment. The animals were identified by making different cuts on the ears which were written on the tags of that respective cut and were placed in individual cages. The two treatments were as follows:

1. Non-irradiated (control)

2. Irradiated (Experimental)

Preparation of diets for feeding animals

The animals were fed with non-irradiated and irradiated health mix. As the health mix (soya mix) samples were in powdered form, they were mixed with enough warm water, salt and steamed. After steaming the diets were kept for cooling and then fed to the experimental animals. Boiled and cooled water was also provided in feeding bottles along with the feed.

Experimental period - The feeding experiment was carried out for Five weeks. The amount of feed given and the leftover feed were recorded each day. The growth of animals was monitored by taking their weighs twice a week. After completion of the experimental period the animals were sacrificed.

Termination of the experiment

At the termination day of the experimental period all the albino rats are weighed and noted. The tail of rat tip was cut and blood was collected for the determination of blood haemoglobin level by the method of cyanmethaemoglobin. The rats were dissected and viscera of rat were observed.

Liver of the rat was taken weighed and noted. Bone was taken out and measured the length and weight of the bone, Liver wet was weighed and dried it for further analyses. The dissected rat was shown in Fig. 1.

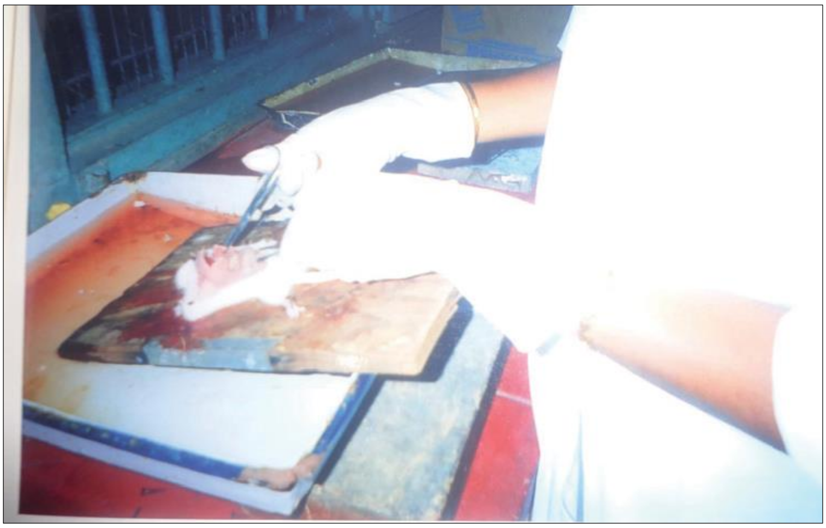

Fig. 1: Dissected Rat

Analysis of the Liver

The liver wet and dry weight, liver and fecal nitrogen were analyzed by the procedure followed by AOAC (Horwitz, 1975). Triplicate samples of 100mg each of the powdered liver and fecal samples were taken for analysis.

The various nitrogenous compounds in the ground sample were converted to Ammonium Sulphate by boiling with concentrated H_2SO_4. It is subsequently decomposed by addition of excess alkali and the liberated ammonia is absorbed into boric acid solution containing methyl red indicator by steam distilling. Ammonia forms a loosed compound ammonium borate with boric acid which is titrated directly against standard H_2SO_4 (0-7N).

Determination of Blood Haemoglobin

Capillary blood samples are used for haemoglobin estimations by using Cyan ethaemoglobin method. The technique is skin puncture and sample collection is of prime importance as incorrect methodology leads to inaccurate results.

Statistical Analysis

The data obtained on liver and fecal protein levels, bone weight and length changes, haemoglobin level were tabulated. Statistical analysis was carried out, Paired ‘t’ test and ANOVA by using SPSS were carried out to test the paired significant difference between various tests.

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3. RESULTS AND DISCUSSION

The results obtained for selected parameters of albino rats are liver wet and dry weight , bone length and weight and Hemoglobin to observe the growth factors of Albino Rats fed with Non-irradiated and Irradiated Soya Mix for five weeks were discussed. The results obtained for liver wet and dry weight and bone length and weight for non-irradiated and irradiated diet fed albino rats are tabulated in Table 1.

TABLE 1: Mean Values of selected Parameters of Albino rats fed with Non-irradiated and Irradiated Soya Mix

Parameters	Mean ± SD		't' Value
	Non-irradiated	Irradiated	
Liver(Wet) Weight (g)	3.468 ± 0.304	3.113 ± 0.59	1.314*
Liver(Dry) weight (g)	0.970 ± 0.089	0.953 ± 0.204	0.212*
Bone length (cm)	0.783 ± 0.388	0.863 ± 0.083	-0.427*
Bone weight(Wet) (g)	0.285 ± 0.043	0.308 ± 0.011	0.134*

*- Significant @ 1% level

The date in table 1 shows that bone length and bone weight was increased in irradiated soya mix then the rats fed with non-irradiated soya mix. This clearly indicates that availability of calcium was more in soya mix. By maintaining adequate calcium intake during childhood is necessary for the development of a maximal peak bone mass. The primary surrogates used are optimization of calcium balance or achievement of greater bone mass in children was increased with calcium intake (Miller & Weaver, 1994). Calcium is an important basic nutrient for bone synthesis and shape.

The liver wet and dry weight shows a slight decrease in irradiated samples when compared with the non irradiated sample. The difference between the irradiated and non-irradiated soya health mix was found to be significant @ 1% level. The collected liver and bone samples were presented in Fig. 2.

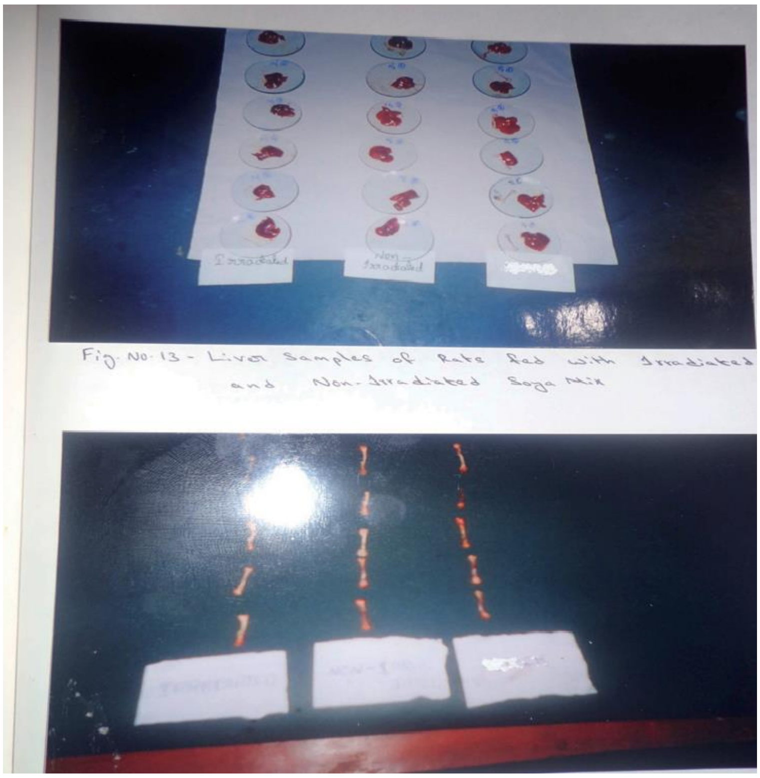

Fig.2: Liver and bone samples of albino rats after dissection

Liver Nitrogen and Faecal Nitrogen and Haemoglobin Albino Rats Fed with Non- Irradiated and Irradiated Soya Mix

Liver is a vital organ and is a complex largest gland associated with the digestive system. It has several functions in the nutrition well being. It is involved in physiological and metabolic functions of the body, the liver metabolic nutrient (Zaefaraia, 2019).

TABLE 2: Liver Nitrogen and Faecal Nitrogen and Haemoglobin of albino Rats fed with Non-irradiated and Irradiated Soya Mix

Parameters	Mean ± SD		't' Value
	Non-irradiated	Irradiated	
Liver N (mg%)	1.680 ± 1.762	4.853 ± 1.309	-3.549*
Faecal N (mg%)	2.210 ± 0.696	2.566 ± 0.838	-0.805**
Hb (g%)	13.40 ± 3.345	13.98 ± 3.01	-0.223*

* : Significant at 1% level

** : significant at 5% level

Table 2 shows the liver nitrogen, faecal nitrogen and haemoglobin percentage of albino rats fed with non-irradiated and irradiated soya mix. The non-irradiated diet albino rats gain less percentage of liver, faecal and haemoglobin % when compared with irradiated diet. Protein synthesis takes place in liver, it is expected that quality and quantity of protein from diet may influence liver weight and also protein level.

Various studies conducted by Eusebio (1996) on liver nitrogen stores of weanling albino rats fed with varied levels, and quality of protein revealed that the protein stores are depicted in malnourished conditions and a higher level of good quality protein level of the rats after feeding for five weeks.

Iron and protein are essential for the haemoglobin formation, the formulated soya mix feed rats shows increase in protein and Hb%. Kokel (1996) studied on the influence of dietary protein on the Hb% and growth of young albino rats by feeding the two different diets with 35, 52 and 70 g protein diet and without protein diet which were fed to different groups and their Hb levels are observed. The results showed that Hb% is high in protein rich diets.

4. SUMMARY AND CONCLUSION

The need to preserve food has been felt by mankind since time immemorial. Radiation processing of food is one of the latest methods developed for this purpose. Irradiation, carried out under conditions of good manufacturing practice, is recommended as a safe and effective food processing method that can reduce the risk of food poisoning and preserve foods without any detrimental to health and with minimum effect on nutritional quality. Soy protein is the only vegetable source of complete protein. Indian diets were mainly based on cereals and pulse combination. The formulated soya mix is also combination of cereals and pulses; nutritionally this combination is good for health. The animal experiment of formulated soya mix clearly shows that more percent of calcium, haemoglobin and protein percentage, the irradiated soya mix shows good result than the non irradiated soya mix. The irradiated health mix increases the shelf life and also retain good amount of nutrients which helps for growth and development.

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