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Electron beam irradiation reduces the anti-nutritional factors from plant based aqua-feed ingredients

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ABSTRACT

The effect of electron beam (EB) radiation on the anti-nutritional factors (ANFs) of unconventional plant based feed ingredients such as cotton seed cake; rubber seed cake and soybean meal were studied. All the three ingredients were exposed to 0 (control), 5, 10, 15 or 50 KGy of electron beam. The anti-nutritional factors examined were phytic acid, tannin and hydrocyanic acid. The results revealed that EB irradiation significantly reduced ($P<0.05$) the phytic acid, tannin and hydrocyanic acid content in all the three ingredients. The degradation of ANFs varied with respect to dose of electron beam radiation and the feed ingredients as well. Using broken line analysis it was found that a radiation of 9.33 KGy was found to be optimum to reduce hydrocyanic acid, phytic acid and tannic acid to the extent of 34, 63, and 22%, respectively. This irradiation method appears to be user friendly compared to traditional methods for reducing the ANFs present in the plant ingredients.

Key words: Electron beam radiation, anti-nutritional factor, phytic acid, tannin, hydrocyanic acid.

INTRODUCTION

Aquafeed industry has shown consistent growth of around 8% during the last two decades globally. This is due to increased consumption of fish among the health conscious population which inclined towards consumption of quality animal protein. The global animal feed production is 954 million tons out of which 34 million tons (3.6%) are contributed by aquafeed industry [3]. The projected global aquaculture production by 2021 is 79 million tons [6] considering the current growth rate. To fulfill the projected target, aquafeed industry demands almost three fold increase of aquafeed by 2021, which requires infrastructure as well as availability of raw ingredients. The increasing price of feed ingredients and increasing manufacturing and transportation costs are, therefore, likely to have a huge impact on global production and the price of aquafeeds. As the feed ingredients are also used in other livestock feed, the conventional feed ingredients are becoming scarcer day by day. For cost effective aquafeed it is imperative to use unconventional feed ingredients. Generally unconventional feed ingredients have the limitation to be included in the aquafeed due to the presence of ANFs [10]. Effective methods to remove these ANFs facilitate their use in aquafeed. Though many methods are available most of them are not in use due to cumbersome techniques. Hence a user friendly technique to destroy or remove these ANFs from the unconventional feed ingredients is inevitable for development of cost effective aquafeed.

In the present study rubber seed cake, cotton seed cake and raw soybean meal were selected for irradiation as ANFs are high in these ingredients. Rubber seed cake and cotton seed cake are produced after extracting the oil from it which is available in plenty in local market. Soybean meal is easily available and commonly used as feed ingredient after given a heat treatment. Phytic acid, tannin and hydrocyanic acid are the three most dominating ANFs present in these unconventional ingredients in addition to other antinutrients. Though many traditional methods (thermal process, soaking, germination, dry heat) are available for reducing the ANFs but most of these are not user friendly. EB irradiation has been proved to be successful in decontamination, disinfestations and improvement of overall qualities of food and agricultural commodities [14, 5, 11]. Radiation processing has also been shown to reduce or inactivate some of the ANFs in wild leguminous seed and, thereby enhancing their edibility [16, 13]. Information pertaining to nutritional, anti-nutritional and safety characteristics of unconventional aqua-feed ingredients will be a basic necessity, prior to addition to fish feed and commercialisation. The main aim of the present study is to ascertain the impact of EB irradiation on the anti-nutritional component of some locally available potential unconventional fish feed ingredients. The result of the present study intends to provide information for the successful utilisation of electron beam irradiated unconventional feed ingredients in fish feed in order to reduce the feed cost.

MATERIALS AND METHODS

Sample preparation and irradiation
Ingredients such as rubber seed cake, cotton seed cake and soybean meal were selected for electron beam irradiation. EB radiations emitted from the Microtron were passed through the ingredients. The samples were irradiated with 5, 10, 15, 50 KGy EB radiations. The sample without any irradiation was served as control.

Freed ingredients were packed in a specially designed bi-axially oriented polypropylene bags (BOPP, 25 μ, 6 × 6 cm) and exposed to EB radiation at the Microtron Centre, Mangalore University, India to doses of 5, 10, 15 and 50 KGy at room temperature (25±1℃). The dose rate of the electron beam was 500 Gy/min**.** Irradiation was carried out by exposing both sides of the feed for uniformity. The absorbed dose was measured using a Current Integrator (connected to a Fast Current Transformer in the beam transport line) which was calibrated against appropriate dosimeters.

Estimation of ANFs
Phytic acid
Phytic acid was extracted from the finely ground samples and determined by adapting standard procedures [19]. About 2 g of sample was extracted with 1.2% HCl (10 ml) containing sodium sulphate (10%) for 2 hr at room temperature (25±1℃) and centrifuged. The volume was made up to 10 ml with the extracting solvent. Phytate phosphorus was estimated before and after precipitation of phytic acid by $FeCl_3$. Five ml of the above extract was taken to which 3 ml of $FeCl_3$ solution ($FeCl_3$, 2g + concentrated HCl, 16.3 ml, diluted to 1 L) was added, stirred and boiled for 75 min in a boiling water bath, cooled and left at room temperature (1hr) prior to centrifugation (2000 rpm, 10 min) and filtration (Whatman No.1). The supernatant made up to 10 ml with distilled water was used for the assay. Analysis of soluble phosphorus was done by the method described by Bartlett [7] using ammonium molybdate reagent. The absorbance was recorded at 430 nm after 30 min with KH_2PO_4 as standard. Phytate phosphorus was determined by the following formula:

Phytate phosphorus = A × 28.18 / 100

Where A = Phytic acid

Hydrocyanic acid
Hydrocyanic acid content of feed was estimated by the method of AOAC [4]. About 10-20 g samples were taken and finely ground to which 200 ml water was added. It was kept for 2-4 hrs followed by steam distillation. The distillate (150-160 ml) was collected in a beaker containing about 15 ml NaOH solution (2.5%) and the volume was made up to 250 ml. From this 10 ml was taken to which 8 ml NH_4OH (6N) and 2ml KI (5%) were added. Finally it was titrated with (0.02 N) $AgNO_3$. End point is the appearance of a giant, permanent turbidity, especially against black background where,

1ml of 0.02N $AgNO_3$ = 1.08 mg hydrocyanic acid

Tannin
Tannin was estimated using standard folin-denis method [17]. Exactly 0.5 g of the finely ground sample was weighted and 75 ml water was added to it. The flask was heated gently and boiled for 30 min. It was then centrifuged at 5000 rpm for 20 min and then supernatant was collected in a 100 ml volumetric flask and the volume was made up to 100 ml. Then 0.5 ml of the sample extract was transferred to a flask containing 7.5 ml water and 0.5ml of Folin- Denis reagent to which 1 ml Na_2CO_3 solution was added and diluted to 10 ml with water followed by shaking. The absorbance was noted at 700 nm after 30 min. A blank was prepared instead of sample. A standard curve was prepared and tannin was estimated.

Total free amino acid (TFA)

TFA was estimated by using Moore and Stein [18] procedure. Exactly 0.5 g finely ground sample was taken for making a homogenate to which 5-10 ml of 80% ethanol was added, filtered, centrifuged and saved the filtrate. Extraction was repeated twice with the residue and pooled all the supernatants. 0.1ml extract was taken and 1 ml ninhydrin solution was added. The volume was made up to 2 ml with distilled water. Tubes were heated in boiling water bath for 20 min and 5 ml diluents were added followed by mixing. After 15 min reading was taken at 570 nm.

Statistical analysis

The mean values were analyzed by using the statistical software SPSS version 14.0. Mean values between treatments were compared using Duncan multiple range test. Difference were considered at significance of ($P<0.05$).

RESULTS AND DISCUSSION

Reduction of ANFs

Phytic acid

The phytic acid content of different feed ingredients ranged from 355.79 to 883.89 mg/100g with highest in soybean meal (table1). The reduction of phytic acid increased with the increase of the irradiation doses. The breakdown of phytic acid by EB radiation may be attributed to cleavage in the structure or to the formation of inositol phosphate due to the action of free radicals generated during radiolysis [2]. Maximum reduction of phytic acid (84.55%) was recorded in soybean meal followed by rubber seed cake (Fig1). However, only 48.84% of reduction was found in the cotton seed cake. This indicates that EB irradiation was not that much effective in reducing the phytic acid content in cotton seed which may be due to the presence of high fiber. The optimum doses of the electron beam to reduce the ANFs appear to be ingredient specific, where maximum reduction was found in soybean meal than the others. Bhat et al. [15] reported complete elimination of phytic acid at 5 KGy EB radiations without affecting the nutritional quality of lotus seed. Similar observation was also reported by many authors in different bean viz 21% reduction of phytic acid in carioca beans and 14% reduction in masacar bean exposed to 5 KGy EB radiation [1] and 15.25% in broad bean when exposed to 10 KGy gamma radiations [12].

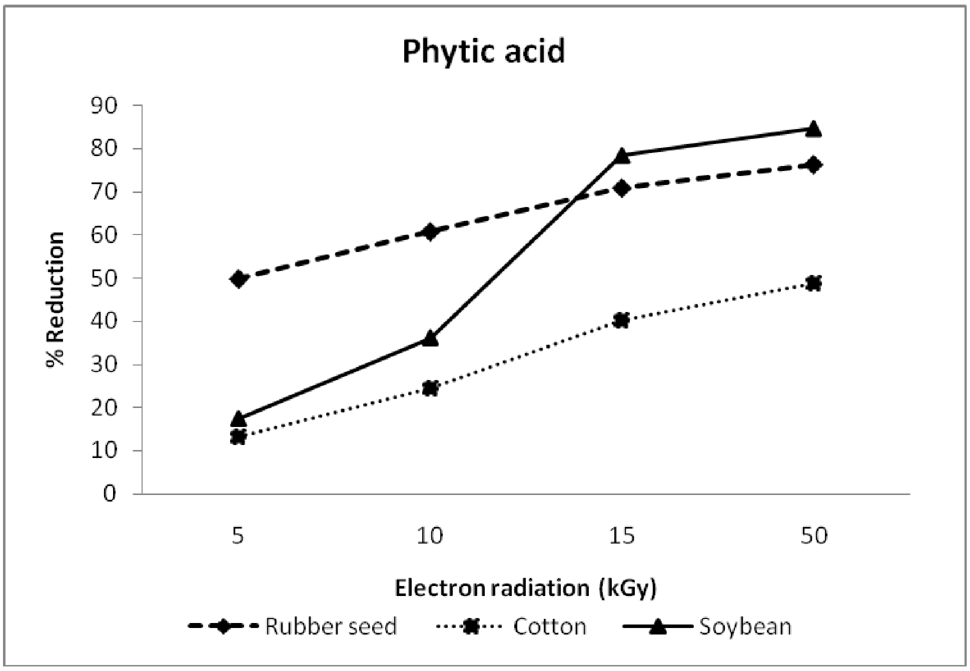

Fig 1. Reduction of Phytic acid (%) due to exposure to electron beam radiation

Hydrocyanic acid

The hydrocyanic acid of the ingredients ranged from 2.69 - 3.2 mg/100g feed with highest in rubber seed cake (table 1). Hydrocyanic acid content in all the ingredients reduced significantly ($P< 0.05$) with increased irradiation level up to 50 KGy. Maximum reduction of hydrocyanic acid (%) among the three ingredients was found in rubber seed cake (fig 2). Hence, EB irradiation can be considered more effective to reduce hydrocyanic acid content of rubber seed cake than the cotton seed cake and soybean meal. In soybean meal 50 KGy radiation exhibited highest reduction of hydrocyanic acid. However, above 5 KGy the percentage reduction of hydrocyanic acid was not significantly different from other irradiation doses. Hence, 5 KGy radiations appear to be optimum to reduce the hydrocyanic acid in soybean meal. The efficacy of EB irradiation showed different trend to reduce the hydrocyanic acid in rubber seed cake. 15 KGy and 50 KGy radiation reduces 42.5% and 65% hydrocyanic acid respectively of rubber seed cake (Fig 2) which are not significantly different (P>0.05). Hence, it indicates that 15 KGy is optimum to reduce the hydrocyanic acid in rubber seed cake. Cotton seed cake registered maximum reduction of hydrocyanic acid at 50 KGy radiation which indicates that EB irradiation is not that much effective in reducing the hydrocyanic acid in cotton seed cake. Results from this study revealed that EB irradiation can easily break down the hydrocyanic acid content in soybean meal compared to rubber seed cake and cotton seed cake.

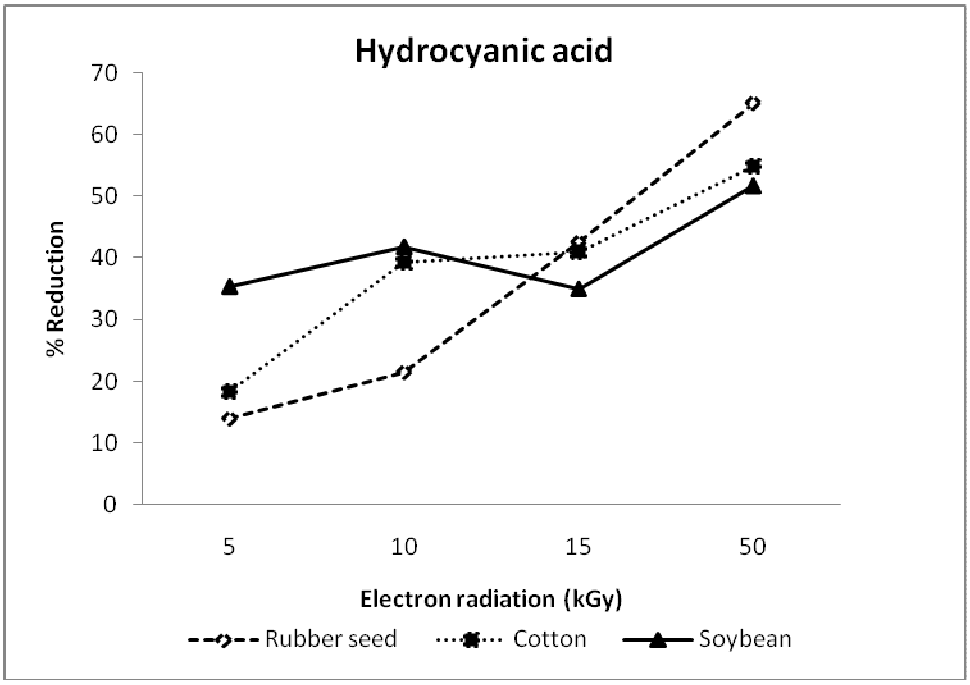

Fig 2. Reduction of Hydrocyanic acid (%) due to exposure to electron beam radiation

Table 1. Phytic acid (mg Phytic acid /100g of sample) and hydrocyanic acid (mg HCN/100g of sample) content of different feed ingredients exposed to electron beam radiation.

Treatments Irradiation (KGy)	Rubber seed cake		Cotton seed cake		Soybean meal	
	Phytic acid	Hydrocyanic acid	Phytic acid	Hydrocyanic acid	Phytic acid	Hydrocyanic acid
0	443.38±10.35^{d}	3.20±0.25^{c}	355.79±13.12^{d}	2.69±0.16^{d}	883.89±55.11^{c}	3.16±0.16^{b}
5	222.69±18.25^{c}	2.75±0.19^{c}	308.90±14.33^{c}	2.20±0.18^{c}	729.59±83.90^{b}	2.04±0.18^{a}
10	174.41±10.35^{bc}	2.51±0.23^{bc}	268.90±20.04^{b}	1.63±0.09^{b}	564.07±56.56^{b}	1.84±0.09^{a}
15	129.58±3.45^{ab}	1.84±0.09^{ab}	212.35±2.75^{a}	1.58±0.05^{b}	191.66±13.45^{a}	2.05±0.05^{a}
50	105.45±10.65^{a}	1.12±0.09^{a}	182.00±9.65^{a}	1.21±0.08^{a}	136.48±13.79^{a}	1.54±0.08^{a}

Mean value in the column with different superscript differ significantly (P<0.05). Data expressed as mean ±SE

Tannin

The tannin content of the ingredients ranged from 5.00 - 6.79 mg/100g feed with highest in cotton seed cake (table 2). Maximum reduction of tannin for all the ingredients was recorded at 10 KGy radiation (fig 3) suggesting 10 KGy radiation is optimum to reduce the maximum level of tannin content in various feed ingredients. Toledo et al. [20] also reported a maximum reduction of tannin in soybean meal exposed to 8 KGy of gamma radiation. Villavicencio et al. [1] reported similar to our study where tannin content of carioca and macacar seed decreased with EB radiation at 10 KGy. Whereas, Bhat et al. [14] reported the complete reduction of tannin of lotus seed at 5 KGy EB radiations. Unlike cotton seed cake, rubber seed cake exhibited somewhat resistant to tannin degradation when exposed to EB radiation. This indicates that EB irradiation is ingredient specific to reduce the tannin also.

Total free amino acid (TFA)

The initial TFA was found maximum in rubber seed cake followed by soybean meal and lowest value found in cotton seed cake (table 2). Maximum production of TFA for all the three ingredients was found at 10 KGy radiations. Cotton seed cake showed highest production of TFA at 10 KGy radiations which was significantly different from other treatments, indicating the positive impact of EB irradiation on TFA of cotton seed cake.

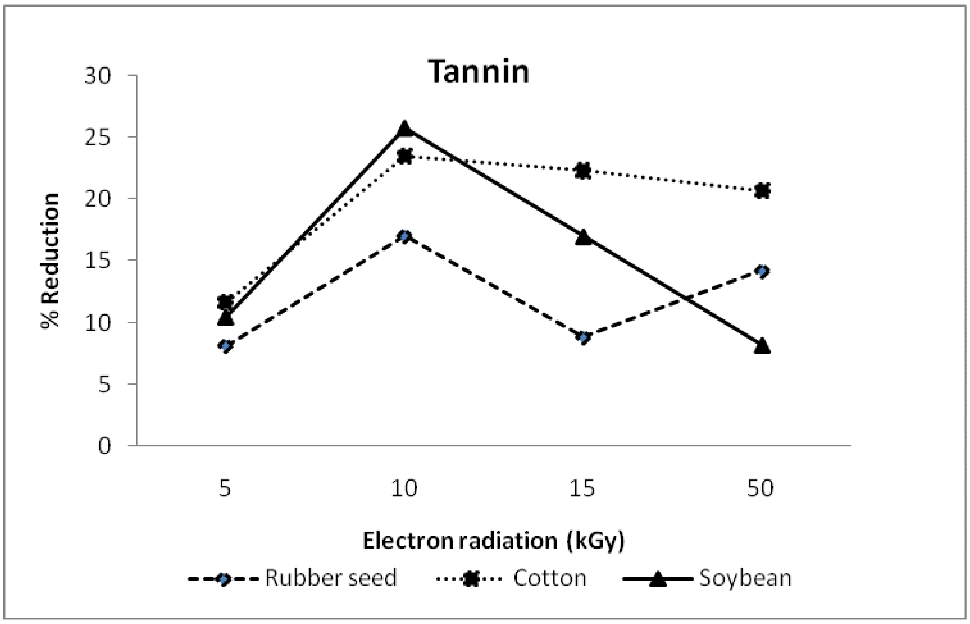

Fig 3. Reduction of Tannin (%) due to exposure to electron radiation

Table 2. Tannin (mg tannin/100g of sample) and total free amino acid (µg /100g of ample) content of different feed ingredients exposed to electron beam radiation

Treatments Irradiation (KGy)	Rubber seed cake		Cotton seed cake		Soybean meal	
	Tannin	Free amino acid	Tannin	Free amino acid	Tannin	Free amino acid
0	5.08±0.15[bc]	6.81±0.14[a]	5.54±0.15[c]	1.06±0.09[a]	6.79±0.17[b]	3.45±0.11[a]
5	4.66±0.29[b]	8.66±0.23[ab]	4.89±0.29[b]	1.37±0.26[a]	6.08±0.10[ab]	3.92±0.14[b]
10	3.9±0.23[a]	9.86±0.39[b]	4.24±0.32[a]	2.41±0.11[b]	5.05±0.47[a]	3.92±0.08[b]
15	4.63±0.25[b]	8.57±0.56[ab]	4.31±0.25[a]	1.25±0.30[a]	5.64±0.46[ab]	3.92±0.17[b]
50	4.36±0.13[a]	8.13±0.24[ab]	4.39±0.13[a]	1.46±0.30[a]	6.24±0.35[ab]	3.92±0.11[b]

Mean value in the column with different superscript differ significantly (P<0.05). Data expressed as mean ±SE.

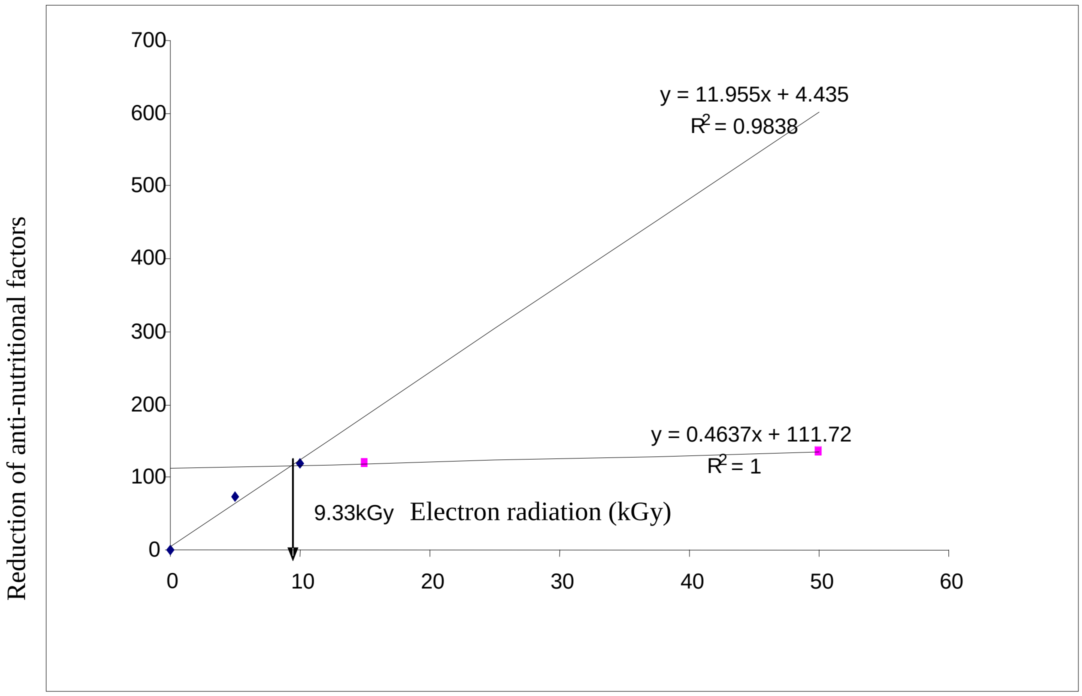

Fig 4. Broken line graph for optimizing irradiation dose

The reduction of different ANFs appears to be ingredient and dose specific. But it is practically impossible to give multiple irradiations to reduce many ANFs present in one ingredient. Hence selecting a single dose which can reduce the many ANFs to maximum extent is of paramount importance for practical point of view. In this context a broken line (fig 4) analysis revealed that when total reduction (%) of hydrocyanic acid, phytic acid and tannin were pooled together an optimum level of 9.33 KGy found to reduce all the ANFs to the maximum level.

CONCLUSION

Result from this study suggested that EB irradiation can be opted as a simple and user friendly method to reduce the ANFs in fish feed. 9.33 KGy EB radiation can be considered as the optimum level to reduce tannin, phytic acid and hydrocyanic acid from unconventional aqua-feed ingredients. As a physical process, EB irradiation definitely has an upper hand against conventional methods in the reduction of anti-nutrients. However, the impact on nutritional qualities needs to be studied. This is probably the first study of its kind where possibility was visible to reduce many ANFs of the plant ingredients in a user friendly way.

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