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Electron beam irradiation on Aquafeed: Effect on growth and in vitro protein digestibility in *Labeo rohita* and *Clarius batracus*

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ABSTRACT

In vitro protein digestibility study of electron beam irradiated (10KGy) unconventional feed ingredients such as cotton seed cake, rubber seed cake and soybean meal and complete diets were conducted on Labeo rohita and Clarius batracus. Complete diet without irradiation served as control. Gut crude enzyme extracted from the experimental species were used to assay in vitro protein digestibility. Electron beam irradiated diet showed significant reduction of phytic acid and tannin content at 10 KGy radiation. All the irradiated ingredients perform higher values of digestibility than the nonirradiated ingredients on both the species. The apparent in vitro digestibility of protein in irradiaited diet were 85.2%, 91% respectively for Labeo rohita and Clarius batracus which were significantly higher than the control diet. A feeding trial was conducted for 45 days to study growth, survival of Labeo rohita fingerlings fed with electron beam irradiated diets. Significantly higher weight gain, SGR and better FCR (1.55) were observed for fingerlings fed with electron beam irradiated diet. The study concludes that the better growth and FCR in irradiated diet fed group may be due to increased bioavailability of protein by electron beam irradiation.

Key words: Antinutritional factors, Protein digestibility, Electron beam irradiation, *Labeo rohita*

INTRODUCTION

Aquaculture is one of the fastest growing food producing sectors with a projected global production of 72 mt by 2021 [8]. To fulfil the projected target, aquafeed industry demands almost three fold increase of aquafeed by 2021 [8]. Feed cost and feed efficiency are among the prime factors that control the farm economy. Economically productive aquaculture systems depend upon an adequate supply of low cost feeds with high nutritional quality. The major operational cost in the aquaculture is feed which is contributing to about 40%-60% of total cost [7] in fish culture. Animal protein sources like fishmeal used in aquafeed are more expensive and scarce than plant protein sources. Thus it is necessary to incorporate cost effective and locally available dietary feed ingredients in order to reduce feed cost [6].

Locally available feed ingredients such as soybean meal, rubber seed cake, cotton seed cake are the potential source of energy, but considered as unconventional feed ingredients. These plant based ingredients have limited use in aquafeed due to presence of anti-nutritional factors (ANFs) such as phytic acid, tannin, hydrocyanic acid etc. AFNs present in unconventional feed ingredients make complexes with proteases and amylases of the intestinal tract, thereby inhibiting the digestibility and reduce proteolysis [24]. Phytate or phytic acid has been reported to reduce protein digestibility and limit the bioavailability of minerals [22] Many traditional methods such as thermal process, soaking and dry heat are reported for reducing the ANFs in feed ingredients [26]. But this method has limitation such as inability to completely remove ANFs and lack of digestibly by targeted organism. Use of electron beam (EB) radiation can be considered as an emerging technology for the elimination of ANFs in the dietary ingredients.

Free- electron is one of the newest dimensions of irradiation of agricultural products. The high- energy electron beam which once passed through materials brings about physical, chemical and biological changes. EB irradiation has been found to be successful in decontamination, disinfestations and improvement of overall qualities of food and agricultural commodities [5, 25, 31]. EB irradiation has been found to be effective in improving protein quality of soybean, oil seed meal and broad bean and thereby increasing its edibility [23, 16, 28, 1]**.**

However, there is huge potential for the unconventional feed ingredients in animal nutrition, especially in aquafeed. Use of these ingredients in fish feed after proper processing through EB radiation can be effective for producing cheap and nutritionally sound aquafeed. India with highest fresh water aquaculture production from carps has huge potential to utilize irradiated ANFs free and cheaper ingredients in carp diets. But prior to their addition into fish feed and commercialisation there should be having basic information regarding nutrients, antinutrients and safety characteristics of these ingredients after being irradiated with EB radiation. With this view point a preliminary study was designed to study the effect of EB irradiated feed on growth and protein digestibility in *Labeo rohita* (*L. rohita)* and *Clarius batracus* (*C. batracus*).

MATERIALS AND METHODS

2.1. Feed formulation and irradiation

A feed was formulated with 33% protein using ingredients such as soybean meal, cotton seed cake and rubber seed cake, corn flour and the feed formulation was given in Table 1. All the ingredients were weighed properly and were then mixed to form dough with the addition of the necessary quantity of water and finally incorporated with oil and mixed well. The dough was cooked for 30 min and was added with vitamin and mineral premix. Dough was pelleted using hand pelletizer and was air dried for 1-2 hrs and kept in hot air oven at 50^0C over night for complete drying and was stored in room temperature. After drying, pellets were packed in two polythene bags, one bag was given 10 KGy EB irradiation (treatment), while other bag was not given any irradiation (control) and sealed airtight. Meanwhile, the feed ingredients viz soybean meal, cotton seed cake and rubber seed cake were irradiated at 10KGy EB radiation individually to perform the in vitro protein digestibility study of the individual ingredients in *L. rohita* and *C. batracus*.

Table 1. Composition of experimental diets t used in feeding trials of *L. rohita* fingerlings for a period of 45 days

Ingredients	Percentage (%)
Soya meal	40.0
Cotton seed	22.5
Rubber seed	22.48
Corn flour	5.0
Cod liver oil	6.0
Vitamin and mineral mixture	2.0
CMC	2.0
BHT	0.02

Composition of vitamin mineral mix (Agrmin) (quantity/kg)

Vitamin A-6,25,000 IU; Vitamin D_3-62,500 IU; Vitamin E-250mg; Nicotinamide-1g; Cu-312mg; Co-45mg; Mg-6g; Fe-1.5g; Zn-2.13g; I-156mg; Se-10mg; Mn-1.2g; Ca-247.34g; P-114.68g; S-12.2g; Na- 5.8mg; K-48.05mg.

2.2. Proximate analysis of experimental diets

All the ingredients were homogenized and the proximate composition of the experimental diets were analysed as per the standard methods of AOAC [4]

2.3. Estimation of anti-nutritional factors of experimental diets

Phytic acid and tannin content of the ingredients and the complete diets were measured (Table 3). Phytic acid was extracted from the finely ground samples and determined by adapting standard procedures [34]. Tannin was estimated using standard folin-denis method [33].

2.4. In vitro Digestibility Studies

2.4.1. Preparation of enzyme extract

Live specimens of *L. rohita* and *C. batracus* (35 g±1.5g) were collected from local market and were acclimatized and reared on control diet in the tubs (57 X 36 X 47 cm, 75 L capacity) for two weeks before sampling for enzyme extraction. After 14 days, the fishes were dissected and the gut contents were cleaned and the intestine was homogenised (1:3w/v) in 50mM tris buffer (pH 8.0) containing 200mM NaCl at 4°C. The homogenate was centrifuged at 10000 rpm for 60 min at 4°C and the supernatant was dialysed against 10mM phosphate buffer (pH 7.8) over night at 4°C using a dialysis membrane. The dialysed crude enzyme extract was obtained by centrifuging at 10000 rpm for 60 min at 4°C and kept frozen at -80°C until final use.

2.4.2. In vitro protein Digestibility

In vitro protein digestibility of the experimental diets and the feed ingredients were determined using the standard methods described by Hsu *et al.* [14] and Saterlee *et al.* [19]. To the 1g of ground feed, 49 ml of 10 mM phosphate buffer (pH 7.6) and 1ml of previously prepared enzyme were added and mixed thoroughly and was incubated for 30 min at room temperature in a shaking incubator. Added 3 ml (5%) TCA solution to 2 ml aliquot, mixed thoroughly and incubated for 30 min and mixture was centrifuged at 3000 rpm. The supernatant was added with 5 ml of 0.5 N NaOH and 1.5 ml of diluted Folin-Ciocalteu phenol solution and incubated for 10 min. After incubation absorbance was taken at 691 nm. Casein was introduced as a reference protein every time in vitro digestion was performed, as a control of reproducibility. Mean while a tyrosine standard curve was prepared. The amount of tyrosine released due to hydrolysis of the substrate by the enzyme can be obtained from the standard curve which is directly correlated with the digestibility of the substrate.

2.5. Experimental set up for growth study of *L. rohita* fingerlings

The experimental setup consisted of six plastic rectangular tubs (57 X 36 X 47 cm, 75 L capacity) covered with perforated lids. Seventy two fishes (5±0.5g) of *L. rohita* fingerlings were distributed into two treatments in triplicates.

2.6. Feeding trial and sampling

The fishes were fed at 3% body weight with different experimental diets for a period of 45 days. Feeding was adjusted to the biomass after every sampling at 10 days interval and the daily ration was divided into 2 equal parts and fed. Growth of the fishes was evaluated in terms of specific growth rate (SGR), feed conversion ratio (FCR)

FCR: Feed given (dw)/ Weight gain (ww), where dw= dry weight, ww=wet weight

SGR: (ln Fw- ln IW / N) X 100, where FW= final weight, IW=Initial weight, N= No of culture days.

2.7. 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 95% level of significance ($P<0.05$).

RESULTS AND DISCUSSIONS

3.1. Proximate analysis of the experimental diets

The proximate composition of the experimental diets, both irradiated and nonirradiated at 10 KGy radiation are shown in Table 2. Crude protein, crude fat, moisture, and ash content of the experimental diets revealed that the chemical composition of diets were not altered by EB irradiation. The similar observation were reported earlier by El-Neily *et al.* [13] in cotton seed cake where it indicated that gamma radiation below 30 KGy are not sufficient enough to change its composition. Other studies on the effect of radiation process on chemical constituents of feed ingredients indicates that irradiation at doses below 45 KGy did not change the chemical composition of canola meal, whole cotton seeds (Ebrahimi *et al.*, 2009; 28). Whereas, the carbohydrate content in the experimental diet was found to be within the range of 44.94 to 46.31%. The increase in carbohydrates in irradiated diet might be attributed to the breakdown of complex sugars (polysaccharides) into simple extractable forms e.g., free sugars [31]

Table 2: Proximate composition of electron beam irradiated and non-irradiated diet used in feeding trials of *L. rohita* fingerlings for a period of 45 days

Nutrient (%)	Electron beam irradiated feed	Non-irradiated feed
Organic Matter	89.32	89.05
Crude Protein	33.06	33.98
Ether Extract	9.95	10.13
Total Carbohydrate	46.31	44.94
Ash	10.68	10.95
Digestible Energy (Kcal/kg)	407.03	406.85

3.2. Anti nutritional factors

The contents of antinutritional factors of the experimental diets are shown in Table 3. EB irradiated diet recorded significant reduction ($P<0.05$) in phytic acid and tannin content than the nonirradiated diets. Several authors reported that EB radiation at doses of 10 KGy can effectively reduce the antinutritional factors in macacar seed, spray dried blood meal, soybean meal [2, 17, 18].

Table 3. Estimation of phytic acid (mg/100gm) and tannin (mg/100gm) of the experimental diets

	Non-irradiated	Irradiated
Phytic acid	456.67 ^{a} ±0.92	253.22 ^{b} ±0.89
Tannin	7.2 ^{a} ±0.15	3.8 ^{b} ±0.32

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

3.3. In vitro protein digestibility

The in vitro protein digestibility using enzyme extract from *L. rohita* and *C. batracus* of the experimental diets and the feed ingredients like soybean meal, cotton seed cake and rubber seed cake showed significant difference between EB irradiated and nonirradiated group (Table 4).

In the present study higher percentage of protein digestibility was recorded in EB irradiated diet (85.2%) than the non-irradiated diet (76%) in *L. rohita*. *C. batracus* also showed the same trend with higher values in irradiated diets (91%) than the nonirradited diets (77.5%).

The present study obtained an apparent protein digestibility of non irradiated soybean meal as 80.45% which is similar to the report of Eid & Matty [3]. Whereas Degani *et al.* [9] and Ali *et al.* [12] reported lower value for protein digestibility as 69.8% and 76% in carps and climbing perch respectively. In the present study, the EB irradiated soybean meal exhibited higher value of protein digestibility (88.25%) compared to all the reported values. The enzyme extract from *C. batracus* also perform higher protein digestibility (90.35%) in EB irradiated soybean meal which was higher than the value reported by Brown *et al.* [30] and lower than the values reported by Buyukates *et al.* [39]. Evidence suggests that Phytic acid makes complex with dietary protein and reduce its bioavailability [22]. Higher digestibility of irradiated soybean meal can be attributed to the efficient reduction of phytate by the EB radiation, resulting in increased protein digestibility. Digestibility of soybean meal exhibited higher values in *C. batracus* than the *L. rohita* (Fig 1). This can be attributed to the higher ability of *C. batracus* to digest soya protein in comparison to *L. rohita*.

In present study, the protein digestibility of irradiated cotton seed cake in both the species were relatively higher than that recorded in different fish sp such as channel cat fish, red drum, and silver perch [32, 11, 10] and was similar to the values reported by Noreen & Selim [37] in *L. rohita.*

EB irradiated rubber seed cake also performed higher percentage of protein digestibility on both the species (Table 4). The evidence regarding the in vitro digestibility of rubber seed is scanty, and the present finding indicted the possibility for the use of rubber seed cake in fish feed through proper irradiation.

Research on protein digestibility of EB irradiated feed ingredients mostly focused on animal nutrition. El-Niely [15] reported significant increase in protein digestibility in different legumes exposed in 10 KGy radiation using rat. Shawrang *et al.* [28] reported the improvement of digestibility in barley grains in cockerels, when exposed to EB radiation, whereas, the use of irradiated feed ingredients in aquafeed is limited.

Evidence from in vitro digestibility studies indicates that digestion of unconventional protein is limited because of the structural and conformational changes of the protein molecules [21]. Also, digestibility studies have reported that phytate–protein complexes are less soluble and less subjected to attack by proteolytic enzymes than the same protein alone [38]. The partial removal or inactivation of proteinaceous antinutritional factors generates free protein molecules, which increased the accessibility of the protein to enzymatic attack [40] and subsequently improve the digestibility of protein. The apparent improvement of protein digestibility ensured through irradiation may be attributed to impact of irradiation on the anti-nutritional factors present in unconventional feed ingredients which are more sensitive to enzyme action.

Table 4. In vitro protein digestibility (%) of experimental diets and feed ingredients in *L. rohita and C. batracus*

	Labeo rohita		*Clarius batracus*	
	EB irradiated	Non-irradiated	EB irradiated	Non-irradiated
Experimental diets	85.20 ^{a} ±0.40	76.00 ^{b} ±0.09	91.00 ^{a} ±0.04	77.5 ^{b} ±0.10
Cotton seed cake	83.34 ^{a} ±0.02	73.61 ^{b} ±0.03	82.20 ^{a} ±0.05	76.00 ^{b} ±0.05
Rubber seed cake	77.00 ^{a} ±0.05	66.54 ^{b} ±0.03	72.00 ^{a} ±.001	67.45 ^{b} ±0.58
Soybean meal	88.25 ^{a} ±0.02	80.45 ^{b} ±0.09	90.35 ^{a} ±.003	81.00 ^{b} ±0.45

Mean value in the column with different superscript differ significantly (P<0.05). Data expressed as mean ±SE. Values recorded were arcsine transformed for testing the variance.

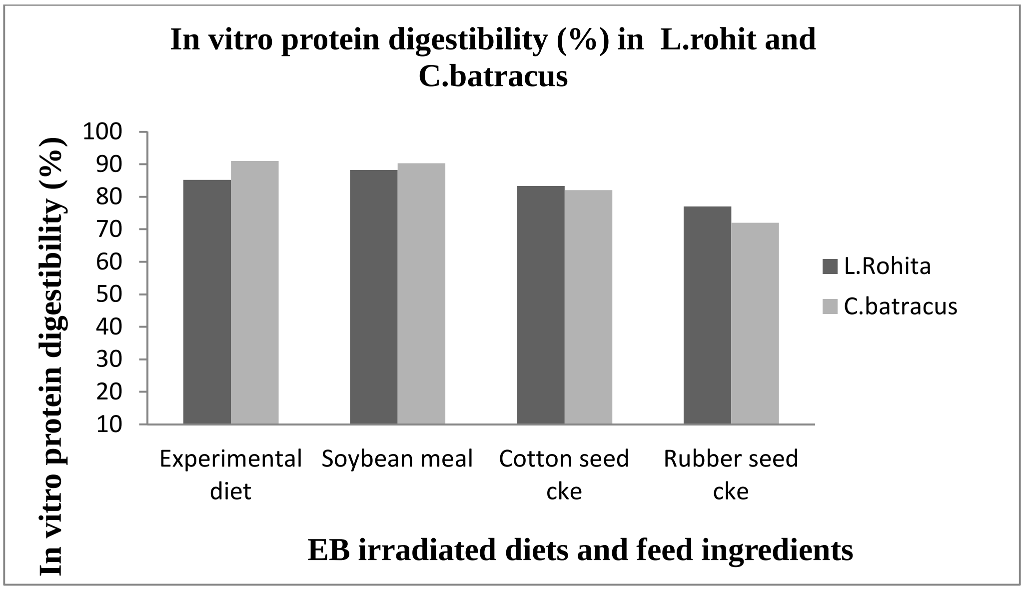

Fig 1. Comparison of invitro protein digestibility (%) of electron beam irradiated ingredients and complete diet in *L. rohita* and *C. batracus*

3.4. Growth parameters

All the groups in the present study recorded 100 % survival indicated that, there is no toxic effect in the diets at 10 KGy EB radiation. Higher weight gain percentage and specific growth rate were recorded for *L. rohita* fingerling fed with EB irradiated diets compared to those fed with non irradiated diets (Table 5). Improved FCR was also recorded in EB irradiated diet fed groups. El-Neily *et al.* [13] reported growth improvement in albino rat fed with cotton seed based diets irradiated with 10 KGy radiation. DeRouchey *et al.* [17] reported that pig showed higher growth rate and better utilization of spray-dried blood meal which processed through EB irradiation.

The difference in growth performance of fish fed with irradiated and non irradiated diet in the present study may be due to presence of antinutritional factors like phytic acid and tannin in the non irradiated diet containing unconventional feed ingredients. Antinutritional factors in dietary ingredients make complex with dietary protein and reduce its bioavailability. Nwanna, *et al.* [20] also reported a negative impact on growth of African Catfish fed with untreated soybean meal of high phytate content. The present findings further concluded that the EB irradiation has positive effect on enhancing the protein digestibility of dietary ingredients by reducing its antinutritional factors in them.

Table 5. Growth parameters of *L. rohita* fingerlings fed with electron beam irradiated feed

Growth parameter	Non-irradiated	Irradiated
Weight gain (%)	54.50 ^{b} ±0.10	62.00 ^{a} ±0.23
[1]SGR	1.58^{b}±0.01	1.82^{a} ±0.10
[1] FCR	1.98 ^{a} ±0.05	1.55^{b} ±0.02
[1]PER	1.56 ^{b} ±0.05	1.85 ^{a} ±0.05
Survival rate ((%)	100.00	100.00

[1]SGR-Specific Growth Rate, [2]FCR -Feed Conversion Ratio, [3]PER- Protein Efficiency Ratio

Mean value in the column with different superscript differ significantly ($P<0.05$). Data expressed as mean ± SE. Values recorded in percentage on wet weight basic were arcsine transformed for testing the variance.

CONCLUSION

Electron beam irradiation offers a good treatment for unconventional feed ingredient to reduce or eliminate their anti-nutritional factors with subsequent increase in their digestibility and thereby, increase the utilization of their proteins in aqua-feed. Therefore, it can be concluded that EB irradiated unconventional feed ingredients have the potential to be used as a source of energy by replacing the conventional feed ingredients in fish feed and thereby, reduces the input cost in aquaculture.

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