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Effect of Gamma Irradiation on Anti Nutritional Factors and Nutritional Value of Canola Meal for Broiler Chickens

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ABSTRACT : Two completely randomized block design experiments were conducted to evaluate the effect of gamma irradiation processing of canola meal on performance parameters of broiler chicks (Ross 308) and protein quality of canola meal. Protein efficiency ratio (PER) and net protein ratio (NPR) were measured as indices of canola meal protein quality. Samples of canola meal were tested for nutritional value after being irradiated at dose levels 10, 20 and 30 kGy. Glucosinolate content was reduced 40, 70 and 89 percent at irradiation dose levels of 10, 20 and 30 kGy respectively ($p<0.01$). Percent of erucic acid in total fatty acid content increased 44, 58 and 48% as a function of radiation dose ($p<0.01$). Dose levels did not affect feed conversion ratio (FCR) and body weight gain of chicks ($p>0.05$). Liver weight was decreased by irradiation dose ($p<0.05$). The same trend was observed for kidney weights, but this trend was not significant ($p>0.05$). Gamma irradiation processing of canola meal had no significant effect on T_3 level in blood of chickens that consumed canola meal, but T_4 level of chicken blood at the 30 kGy dose decreased significantly ($p<0.05$). PER and NPR were not affected by radiation dose level ($p>0.05$). Gamma irradiation seems to be a good procedure to improve the nutritional quality of canola meal. (**Key Words :** Canola Meal, Gamma Irradiation, Performance, Glucosinolate, Erucic Acid, Chickens)

INTRODUCTION

Canola meal (the oil-free residue of low glucosinolate, low erucic acid rapeseed) is a good source of protein for animals and is a particularly rich source of the sulphur containing amino acids, methionine and cystine. Canola meal is characterized as having lower consistent amino acid digestibility and methabolizable energy level than soy bean meal (NRC, 1994). Canola meal has some anti nutritional factors that they are responsible for low utilization of nutrients there are in canola meal. These anti nutritional factors are: glucosinolates, erucic acid, phytic acid and high levels of fiber. High levels of fiber in canola meal are responsible for low metabolizable energy (New kirk et al., 2003). In addition to these anti nutritional factors, the processing conditions affect its quality. For example, extensive heating of oil seed meals during processing can lead to loss in the content and digestibility of amino acids (Parsons et al., 1992).

Removal of undesirable components is essential to improve the nutritional quality of meals and effectively utilize their full potential as animal feed. Several conventional food processing methods such as germination (Nnanna and Philips, 1990; Al-Kaisey et al., 1997), soaking (Jood et al., 1985; Vidal-Valverde et al., 1994), cooking (Sefa-Dedeh et al., 1979; Urbano et al., 1995), fermentation (Zamora and Veum, 1979; Reddy et al., 1980) and gamma irradiation (Rao and Vakil, 1983; Abu-Tarboush, 1998) are known to reduce anti nutritional factors effectively and upgrade the nutritional quality of plant-origin feeds.

However, most of these treatments adversely affect the sensory characteristics of the final product. An additional technique is the application of gamma irradiation, which has already been used for decontaminating food by killing bacteria, insects, and other food born pathogens also to increase the shelf-life of fresh and dry food materials (Farkas, 1988; Molins, 2001; Thorne, 1991). Food irradiation is a physical process involving an energy-input, that does not induce radioactivity in foods. The amount of energy input is called the radiation absorbed dose, and is measured in Grays (1 Gy = 1 J/kg). It is similar in nature to the use of heat via either thermal (infrared) or microwave energies. In contrast to the gross and easily-detectable effects that conventional heat treatments have on foods, the radiation dose generates minute mostly undetectable

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changes in chemical composition (Siddhuraju et al., 2002). Food irradiation has been recognized as a reliable and safe method for preservation of food, improve hygienic quality of foods and improve the nutritional quality of foods (Gampbell et al., 1983; Al-Kaisey et al., 2002; Diehl, 2002). In 1981, the US Food and Drug Administration (FDA) concluded that food irradiated at 50 kGy or less can be considered safe for human consumption (FDA, 1981), and therefore for animal consumption, but irradiation is not well accepted by consumers in several parts of the world.

This study was conducted to evaluate the effect of gamma irradiation on glucosinolates and erucic acid content, and as well to evaluate the effect of gamma irradiation on nutritional quality of canola meal for broiler chickens.

MATERIALS AND METHODS

Radiation processing and analytical methods

Canola meal was packed in polyethylene bags. Each bag contained 1 kg, and these bags were packed in special boxes for irradiation processing (12 bags for each box). These boxes were subjected at ambient temperature to gamma irradiation from a ^{60}Co source (NORDION, IR-136, Canada) at Gamma Irradiation Center, Iranian Nuclear Organization, Tehran, Iran. The applied doses were 10, 20 and 30 kGy as monitored by radio chromic film (McLaughlin et al., 1985). Raw and processed canola meal were stored at 5°C until being used to mix the experimental diets.

Chemical analysis : Chemical composition of canola meal and other feed ingredients were analyzed using AOAC (1990) analytical methods. Glucosinolate content of samples were analyzed with a UV-visible spectrophotometer (Varian, CARY 50 Scan, USA) according to Saini and Wratten (1987). Erucic acid and other fatty acids content of canola meal were measured by a Gas Chromatograph (AGILENT, HP6890, USA) using a capillary column (sge, BPX 70, USA) according to international organization for standardization (ISO 5508, 1990; ISO 5509, 2000).

Plasma hormones analysis : Thyroxin (T_4) and triiodothyronine (T_3) in the plasma samples were assayed by radioimmunoassay (RIA) with commercial Kits (REF: RK-6CT1, Institute of Isotopes, Hungary).

Experiment 1

Day-old commercial male broiler chicks (Ross 308) were fed a conventional corn-soy bean meal diet (Table 1), which was formulated according to the Ross 308 Management Manual (2002). The preliminary feeding period was 10 days. On eleventh day, chicks were wing banded and were individually weighed. A group of 80 chicks of uniform weight was divided randomly in to five groups (a conventional corn-soybean meal diet and 4 test

Table 1. Composition and calculated nutrients content of diets (g/kg) fed in experiment 1 and starter period (0-10 d) of experiment 2

Ingredients	Pre-experiment (0-10 d)	Grower period (10-28 d)		Finisher period (29-42 d)	
		Control	Canola meal	Control	Canola meal
Corn	556.889	620.927	558.860	668.903	606.836
Soybean meal	384.112	336.145	173.172	284.436	121.464
Wheat bran	17.064	-	-	-	-
Vegetable oil	-	5.194	33.579	9.130	37.515
Canola meal	-	-	200.000	-	200.000
DCP	20.370	18.103	17.458	18.695	18.050
$NaHCO_3$	0.789	0.820	0.848	0.823	0.850
$CaCO_3$	9.067	7.924	5.303	7.501	4.880
Common salt	3.000	3.000	3.000	3.000	3.000
Vitamin premix[a]	2.500	2.500	2.500	2.500	2.500
Mineral premix[b]	2.500	2.500	2.500	2.500	2.500
Lysine·HCl	1.971	1.327	2.088	1.110	1.871
Methionine	1.729	1.560	0.692	1.402	0.534
Calculated nutrients content (as-fed basis)					
AME_n (kcal/kg)	2,845	2,965	2,965	3,035	3,035
Crude protein (%)	23	21	21	19	19
Ca (%)	1.00	0.90	0.90	0.90	0.90
Available P (%)	0.50	0.45	0.45	0.45	0.45
Na (%)	0.16	0.16	0.16	0.16	0.16
(Na+K)-Cl (meq/kg)	239.30	218.40	194.20	197.20	173.00
Lys (%)	1.38	1.20	1.20	1.05	1.05
Met+cys (%)	0.90	0.83	0.86	0.76	0.80

[a] Supplied per kg of diet: 22,500 IU vitamin A, 5,000 IU vitamin D_3, 45 IU vitamin E, 5 mg vitamin K_3, 4.5 mg vitamin B_1, 16.5 mg B_2, 25 mg calcium pantothenate, 75 mg niacin, 7.5 mg vitamin B_6, 2.5 mg folic acid, 0.0375 mg B_{12}, 0.25 mg biotin, 625 mg choline and 250 mg anti oxidant.
[b] Supplied per kg of diet: 248 mg manganese, 125 mg iron, 211.75 mg zinc, 25 mg copper, 0.5 mg selenium, and 2.5 mg iodine.

Table 2. Composition and calculated nutrients content of diets (g/kg) fed in experiment 2

Ingredients	Test diet	Nitrogen-free diet
Corn starch	341.703	457.338
Sucrose	335.000	457.000
Vegetable oil	40.000	40.000
Canola meal	243.911	-
DCP	24.961	29.240
$NaHCO_3$	1.341	1.507
$CaCO_3$	5.084	6.915
Common salt	3.000	3.000
Vitamin premix[a]	2.500	2.500
Mineral premix[b]	2.500	2.500
Calculated nutrients content (as-fed basis)		
AME_n (kcal/kg)	3,264	3,703
Crude protein (%)	10.00	0.30
Ca (%)	1.00	1.00
Available P (%)	0.50	0.50
Na (%)	0.16	0.16
(Na+K)-Cl (meq/kg)	97.90	19.50
Lys (%)	0.50	-
Met+cys (%)	0.44	-

[a, b] The same as experiment 1.

diets) with four replicate of four birds. The chicks were allowed *ad libitum* access to feed and water. All groups were kept under control hygienic and environmental conditions. Body weight and feed consumption were recorded at the end of grower (11-28 days) and finisher (29-42 days) periods.

In the isonitrogenous and isoenergetic experimental diets (Table 1) 20 percent canola meal was replaced instead of soybean meal. Five dietary treatments consisted of a control diet and four test diets containing raw and irradiated canola meal (10, 20 and 30 kGy). Experimental diets for grower and finisher period were formulated according to Ross 308 Management Manual (2002). At day 41 two birds of each replicate were chosen randomly for taken blood sample to measure level of T_3 and T_4. At the end of experiment (42 days) two birds from each replicate were slaughtered and carcass, liver and kidneys weights were recorded.

Experiment 2

In this experiment protein quality of irradiated canola meals were evaluated using the PER and NPR bioassay. PER and NPR were carried out according to Trevino et al. (2000). Day-old commercial male broiler chicks (Ross 308) were fed a conventional starter diet, according to Ross 308 Management Manual (2002), (Table 1) from 0 to 7 days post-hatching. After that they were assigned to the dietary treatments. A total of 80 chicks were distributed at random to five treatments, in four replicates of four birds in each. The mean group initial weights were similar (120 g). All the chicks were housed in environmentally controlled starter batteries with raised wire floors. Feed and water were offered *ad libitum* and light was provided continuously. The test diets were fed from 8 to 20 days post-hatching. The feed intake and weight for each replicate were recorded. The five dietary treatments consisted of a protein-free diet and four test diets containing raw and irradiated canola meal (10, 20 and 30 kGy). The test diets were formulated to contain 100 g crude protein/kg, and contain canola meal as a sole source of protein. The composition of experimental diets appears in Table 2.

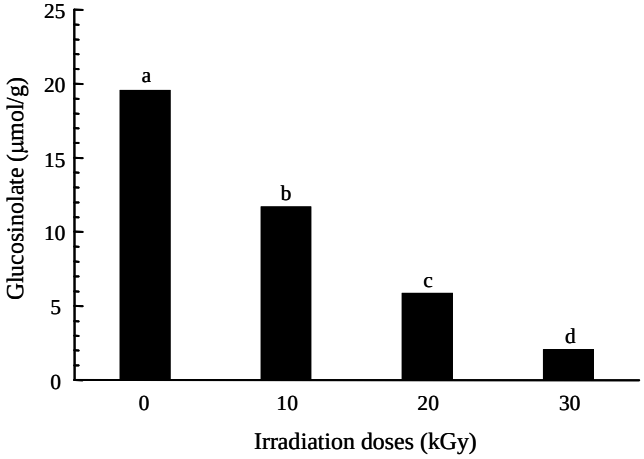

Figure 1. Effect of gamma irradiation on glucosinolate content of canola meal.

PER and NPR were computed by the following formulas:

$$\text{PER} = \frac{\text{Gain body weight}}{\text{Protein consumed}}$$

$$\text{NPR} = \frac{\text{Weight gain of test group - weight loss of protein free group}}{\text{Protein consumed}}$$

Statistical analysis

In experiments 1 and 2, were used completely randomized block design experiment with 5 treatments, 4 replicates per treatment and 4 birds in each replicate. In both experiments, data were analyzed by ANOVA using the General Linear Model (GLM) procedure (SAS institute, 2001). Variables with significant f-tests ($p \leq 0.05$) were compared using Duncan's multiple range test (Duncan, 1955). Differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Effect of gamma irradiation on anti nutritional factors content of canola meal

Glucosinolate : The data presented in Figure 1 show

Table 3. Effect of gamma irradiation on fatty acids composition of canola meal (% in total fatty acid content).

Fatty acid	0 kGy	10 kGy	20 kGy	30 kGy	SEM
C10:0	0.021[a]	0.003[d]	0.012[b]	0.004[c]	0.00000000
C12:0	0.046[a]	0.019[c]	0.043[b]	0.019[c]	0.00000000
C14:0	0.213[a]	0.162[b]	0.199[a]	0.169[b]	0.00158552
C14:1	0.083[b]	0.079[c]	0.086[a]	0.084[ab]	0.00022753
C15:0	0.148	0.154	0.163	0.148	0.00234736
C15:1	0.081[c]	0.080[c]	0.096[a]	0.082[b]	0.00007946
C16:0	7.807[a]	7.343[c]	7.529[b]	7.261[d]	0.00841145
C16:1	2.436[d]	2.559[b]	2.573[a]	2.514[c]	0.00130219
C17:0	0.116[d]	0.124[c]	0.134[a]	0.130[b]	0.00007946
C17:1	0.301	0.342	0.340	0.340	0.00445403
C18:0	1.921[a]	1.664[b]	1.660[b]	1.608[b]	0.00853373
C18:1	25.900[d]	30.307[a]	26.084[c]	27.682[b]	0.01499221
C18:1*cis*	26.704[b]	0.519[d]	27.539[a]	0.594[c]	0.00670896
C18:2*Trans*	0.000[b]	0.000[b]	25.000[a]	0.000[b]	0.00418737
C18:2*cis*	25.792[a]	25.340[b]	0.257[c]	25.322[b]	0.01874662
C18:3*gam*	0.031[b]	0.034[b]	0.041[b]	0.539[a]	0.00223861
C20:0	0.526[a]	0.378[a]	0.537[a]	0.000[b]	0.03497985
C18:3*alph*	5.868[c]	6.062[b]	6.322[a]	6.272[a]	0.01682347
C20:1	0.326[d]	0.378[a]	0.339[c]	0.348[b]	0.00024873
C22:0	0.275[d]	0.325[a]	0.288[c]	0.294[b]	0.00014651
C22:1	0.182[d]	0.262[c]	0.289[a]	0.269[b]	0.00054933
C24:0	0.297[c]	0.361[a]	0.297[c]	0.324[b]	0.00018464
Unknowns	0.920	23.497	0.166	25.989	-

[a, b, c, d] Means in the same row with different superscripts are significantly different ($p<0.05$).

that the glucosinolate content of non-irradiated canola meal was 19.53 μmol/g. The glucosinolate content for canola meals irradiated at 10, 20 and 30 kGy were 11.66, 5.83 and 2.03 μmol/g respectively. The rate of inactivation linearly increased with the increase in irradiation dose ($p<0.01$). The levels of inactivation (as % of raw canola meal) were 40.29, 70.14 and 89.60 percent and were a function of radiation dose.

The above observation indicated that the irradiation treatment has a significant effect on the glucosinolate content of canola meal. Gamma irradiation treatment with its radiolytic effects can destroy glucosinolate molecules. Other investigators reported that anti nutritional factors, such as protease inhibitors (Farag, 1989; Sattar et al., 1990; El-Morsi et al., 1992; Farag, 1998), α-amylase inhibitors (Abu-Tarboush, 1998; Al-Kahtani, 1995), phytohamagglutinins (Farag, 1989; Mahrous, 1992; Farag, 1998), oligosaccharids (Rao and Vakil, 1983; Ghazy, 1990) and tannin (Abu-Tarboush, 1998), significantly inactivated by gamma irradiation.

Erucic acid : According to Table 3, gamma irradiation increased percentage of erucic acid in total fatty acid content of canola meal. Table 3 shows the fatty acid composition in irradiated and non irradiated canola meal. Fatty acid profile of canola meal was changed significantly ($p<0.05$) by irradiation at dose 10, 20 and 30 kGy. Fats are among the least stable feed components being very susceptible to ionizing radiation (Hammer and Wills, 1979). Ismail and Umit (2007) reported that gamma irradiation changed fatty acids composition of food and alteration in fatty acids composition related to irradiation dose. Most of radiolytic products are known to be the same as natural components, but some of these radiolytic products are unique to irradiated feeds. Therefore further studies are need for recognizing these products.

Effect of gamma irradiation processing of canola meal on performance parameters of chicks

Body weight gain, feed consumption and feed conversion ratio were measured as indices of bird's performance. According to data presented in Table 4 body weight gain of birds in different treatments were not affected by dose level of gamma irradiation for canola meal processing ($p>0.05$). Data presented in Table 4 showed that FCR in chicks fed diets containing irradiated canola meal especially in finisher period, were improved. However the difference between means of feed intake and FCR statistically were not significant. Expected improvement in chick's performance due to irradiation decrease the glucosinolate was not appeared. It seems, the reason is that, glucosinolate content of non irradiated canola meal used in present study was low (19.53 μmol/g oil-free residue). Whereas Fenwick et al. (1986) reported that glucosinolate content of rapeseed is more than 30 μmol/g. Canola was developed from rapeseed (*Brassica napus* and *Brassica campestris*) to obtain lower levels of erucic acid (<2%) in

Table 4. Effect of gamma irradiation processing of canola meal on chicks body weight gain, feed consumption and feed conversion ratio in grower (11-28d) and finisher (28-42d) periods

Treatments	BWG (g)		FI (g)		FCR	
	Grower period	Finisher period	Grower period	Finisher period	Grower period	Finisher period
Canola meal 0 kGy	1,002.2	1,130.9	1,563.7	2,297.1	1.56	2.03
Canola meal 10 kGy	978.1	1,096.2	1,557.5	2,202.1	1.59	2.01
Canola meal 20 kGy	965.6	1,151.8	1,493.4	2,241.8	1.54	1.94
Canola meal 30 kGy	954.0	1,165.3	1,515.0	2,218.7	1.59	1.91
Control	996.8	1,138.4	1,551.5	2,291.2	1.55	2.01
SEM	7.454	12.692	12.013	23.390	0.0083	0.013

[a, b] Means in the same column with different superscripts are significantly different ($p<0.05$).

Table 5. Effect of gamma irradiation processing of canola meal on liver and kidney weight (g) of chicks consumed irradiated canola meal

	Liver weight	% liver weight	Kidney weight	% kidney weight
Canola meal 0 kGy	56.31 [a]	2.43	14.06	0.59
Canola meal 10 kGy	46.97 [b]	1.97	13.60	0.58
Canola meal 20 kGy	50.25 [ab]	2.13	12.22	0.51
Canola meal 30 kGy	50.96 [ab]	2.16	12.22	0.52
Control	49.30 [b]	2.08	12.06	0.50
SEM	1.165	0.011	0.251	0.011

[a, b, c] Means in the same column with different superscripts are significantly different ($p<0.05$).

the oil portion and lower levels of glucosinolate (<30 μmol/g) in the meal (Bell, 1993).

According to Summers et al. (1969) high level of glucosinolate in chickens' diet lids to a reduction in feed consumption and growth. Also, leeson et al. (1987) and Ramesh et al. (2006) reported that consumption of low glucosinolate canola meal have not any adverse effect on chickens performance.

According to some studies (Ismail and Osmsn, 1976; Farag, 1989; El-Niely, 1996; Farag, 1998) the gross composition (dry matter, moisture, ash, crude protein, crude fat and crude fiber) of raw and irradiated feed ingredients were not affected by gamma irradiation. Also El-Niely, (1996) has been concluded that moisture content of feed ingredients is the main factor for gamma irradiation effects. It seems that the amount of water in raw canola meal used in present experiments (80.06 g/kg) does not favor the production of enough radiolytic products and water free radicals which needed to induce significant changes in the gross composition of canola meal. Because the gross composition of canola meal was not affected by gamma irradiation, so performance parameters of birds which consumed irradiated canola meals was not affected.

Table 6. Effect of gamma irradiation processing on T_3 and T_4 level in chicks blood (nmol/L)

Treatments	T_3	T_4
Canola meal 0 kGy	2.453	105.11 [a]
Canola meal 10 kGy	2.072	104.63 [a]
Canola meal 20 kGy	2.469	107.14 [a]
Canola meal 30 kGy	2.427	92.99 [ab]
Control	2.005	76.47 [b]
SEM	0.105	2.841

[a, b] Means in the same column with different superscripts are significantly different ($p<0.05$).

Liver and kidney weight

Data presented in Table 5 show that processing of canola meal by gamma irradiation affected the liver weight ($p<0.05$), where by increasing the dose level of gamma irradiation, the liver weight was decreased. The same trend was observed in the case of kidney weight, but this trend was not significant ($p>0.05$).

Summers et al. (1969) showed that high glucosinolate in chick's diet adversely affect performance and have some effect on liver and kidney. In some cases high levels of glucosinolate lead to hemorrhage in liver and kidney. In this study has been observed that by increasing the dose level of gamma irradiation for processing, liver weight and kidney weight were decreased. This reduction in liver and kidney weight might be in result of reduction in glucosinolate content as a function of irradiation dose level.

Thyroid hormones (T_3 and T_4)

The data presented in Table 6 show that gamma irradiation processing of canola meal has not significant effect ($p>0.05$) on T_3 level in chickens blood that consumed canola meal. But T_4 level of chickens blood at dose 30 kGy decreased significantly ($p<0.05$) by gamma irradiation. High levels of glucosinolate can lead to increase in thyroid gland size and also decrease in thyroid hormones level in blood (Chiasson and Sharp, 1979; Bell, 1984).

Because a partial amount of thyroid hormones is T_3, it is

Table 7. Effect of gamma irradiation on canola meal protein quality

Treatments	Initial weight	Weight gain	Protein consumed	PER	NPR
Nitrogen-free diet	126.7	-25.08 [b]	0.00 [b]	-	-
Canola meal 0 kGy	124.0	175.93 [a]	44.85 [a]	3.914	4.480
Canola meal 10 kGy	127.2	170.22 [a]	43.10 [a]	3.948	4.535
Canola meal 20 kGy	125.0	167.84 [a]	42.14 [a]	3.977	4.589
Canola meal 30 kGy	121.3	152.66 [a]	40.03 [a]	3.793	4.440
SEM	0.897	2.438	0.523	0.050	0.054

[a, b] Means in the same column with different superscripts are significantly different ($p<0.05$).

not usually affected by any type of treatment (Karunajaeewa et al., 1990). Then it is logical that T_3 level in chickens blood did not affect by gamma irradiation. But it has been predicted that gamma irradiation by glucosinolate distraction (Figure 1) could increase T_4 level in chickens blood that consumed irradiated canola meal. But it has been seen that gamma irradiation at 10 and 20 kGy did not affect T_4 level and at 30 kGy suddenly it significantly reduced. It can be concluded that T_3 and T_4 level in chicken's blood was not affect by glucosinolate amount. Because glucosinolate content in this variety of canola meal that used in this experiment was low (19.53 μmol/g) and could not affect growth and performance of chickens. Reduction of T_4 level at dose 30 kGy might be related with radiolytic byproducts that were produced at high dose of gamma irradiation.

Effect of gamma irradiation on protein quality of canola meal

To assess whether there was any true improvement in the protein quality as a result of radiation processing of canola meal, PER and NPR assays were carried out on broiler chickens (Ross 308). PER and NPR of raw canola meal were found to be 3.914 and 4.480 respectively. PER values for irradiated canola meals at 10, 20 and 30 kGy dose levels were 3.948, 3.977 and 3.793 respectively and NPR values were 4.535, 4.589 and 4.440 for mentioned dose levels (Table 7). Although gamma irradiation was not significantly affected PER and NPR values of canola meal ($p>0.05$), but by increasing the dose level up to 20 kGy, PER and NPR values were slightly increased and at dose 30 kGy these values decreased. It could be concluded that the amount of moisture in raw canola meal does not sufficient to produce enough free radicals for chemical changes in gross composition of irradiated canola meals.

CONCLUSION

The results presented here indicated that glucosinolate content of canola meal was decreased as the radiation dose increased. Following the glucosinolate content of canola meal, by increasing the dose level of gamma irradiation, the liver weight of chicks was decreased. These finding and observed performance trends propose that, gamma irradiation had a potential for improving nutritional value of canola meal.

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