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Effects of Electron-Beam Irradiation of the Diet on Microbial Population, Intestinal Morphology, Ileal Digestibility and Performance of Broilers

Research Article

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ABSTRACT

A total of 300 one-day old male broilers (Cobb 500) were randomly divided into four treatment groups. The groups comprised of 15 birds each, and were defined by diet and the birds in each group were fed 0 (control), 3, 5, or 7 kGy electron-beam irradiated diets. A total of 5 replicates were performed. The chemical composition of the diet was not affected by irradiation. Irradiation doses of 5 and 7 kGy completely eliminated microbial load in diets (P<0.05) and decreased the total aerobic and coliform counts in ileum and cecum at increasing rate at 14, 28 and 42 days of age (L: P<0.001; Q: P<0.0001). Total coliforms of the ileum decreased with the increased irradiation dose at 21 and 42 days of age (Q: P<0.0001). Lactobacillus counts improved with increased EBI dose, except for lactobacillus in cecum at 42 days of age. There was a linear increase in villus height (duodenum, jejunum, and ileum) with higher irradiation doses at 21 and 42 days of age. Additionally, a linear increase and a decrease in villus height:crypt depth were observed in the jejunum at 21 and 42 days of age, respectively (P<0.05). The villus height:crypt depth of duodenum and jejunum followed a linear increasing rate (Q: P<0.001) with the increased irradiation dose. Feed intake, mortality (all periods), body weight gain, and feed conversion ratio were similar between treatments at 1-14 and 14-28 days of age. Body weight gain, however, increased at a rising rate (Q: P<0.001), and feed conversion ratio decreased at a diminishing rate (Q: P<0.01) with higher irradiation doses during 28-42 and 1-42 days of age. The ileal digestibility of dry matter, organic matter, ether extracts, gross energy, and apparent metabolizable energy increased with the irradiation dose (Q: P<0.001). Our results indicated that electron-beam diet irradiation reduces microbial coliform counts and supports lactic acid producers in the gastrointestinal tract. Moreover, the ileal digestibility of nutrients, body weight gain, and feed conversion ratio improve with irradiation.

KEY WORDS broiler performance, electron-beam irradiation, ileal digestibility, microflora.

INTRODUCTION

Broiler performance is affected by the nutrients content of the diet and intestinal microbial population. A decrease in microbial contamination or a change in diet structure or both, affect broiler performance (Iji *et al.* 2001).

Contamination of diet with pathogenic microorganisms is a major problem in poultry industry. Additionally, pathogenic microorganisms may affect both animal and human health. Several preventive measures, like disinfectants have been used to reduce feed pathogen load, but their efficiency is often limited (Jay *et al.* 2005).

Irradiation is a promising method of sterilization, without modifying crude protein (CP) and amino acid (AA) content of by-products and diets (Al-Masri, 2003).

Gamma rays (GRs) and electron beams are the most commonly used sources of irradiation. During the gamma irradiation process, highly purified Co^{60} produces GRs to reach a stable Ni^{60} state (Satin, 1996). Electron-beam irradiation (EBI) uses accelerators that generate electron beams of energy that are directed toward the product via a magnet (Nieto-Sandoval *et al.* 2000). Electron beams can penetrate products with a 2 to 4-inch thickness, while GRs can penetrate an entire product. Electron-beam irradiation has its advantages over GRs, which include higher dose rate capability, no nuclear waste, and switchable accelerators. Additionally, it can contact the food product from both its top and bottom, leading to a more uniform application and consequently, more effective bacterial elimination. Thus, EBI is thought to have several advantages over gamma irradiation (Satin, 1996).

Electron-beam irradiation reduced up to 99.9% of the feed pathogenic bacteria load (Rodriguez *et al.* 2006) in a relatively short processing time (Kim *et al.* 2006). Siddhuraju *et al.* (2002) reported that irradiation caused physico-chemical changes in feed, which affect nutrient availability in domestic animals. Improved nutrient utilization by irradiation has been reported in pigs (GRs and EBI) (Derouchey *et al.* 2003), goats (GRs) (Mani and Chandra, 2003), and broilers (GRs) (Al-Masri, 2003). Moreover, irradiation potentially reduces protein solubility (Mani and Chandra, 2003), which is critical for poultry. Irradiation of high molecular weight carbohydrates in a solid state causes external bridges breaking (Siddhuraju *et al.* 2002), facilitating the release of nutrients.

This research studied the effect of EBI on pathogen contamination and chemical composition of a broiler diet, as well as the effects of feeding irradiated diets on intestinal morphology, microflora of the gastrointestinal tract, ileal digestibility of nutrients and broiler performance.

MATERIALS AND METHODS

Animals and diets

A total of 300 one-day old male broilers (Cobb 500) were randomly allocated into four treatment groups. Each group was fed a different dose of irradiated feed: 0, 3, 5 and 7 kGy EBI. Each treatment was repeated five times, and the experimental unit was a pen with 15 chicks. Feed (milled) and water was offered for *ad libitum* consumption throughout the study, and the lighting schedule was 23 h light / 1 h dark. The chicks were accommodated at 32 ˚C on the first day. This was subsequently reduced to 3 ˚C each week, until the end of the third week.

Diets were formulated to meet the nutrition requirements of broilers (NRC, 1994). They were packed in 30 × 70 cm polyethylene bags (0.5 mm thick), and exposed to EBI at a dose of 0, 3, 5, and 7 kGy at room temperature. A Rhodotron accelerator (Model TT200, Ion Beam Applications; Chemin du Cyclotron., Louvain-la-Neuve, Belgium) in the Yazd radiation processing center (AEOI, Yazd center, Iran) was used. Cellulose triacetate film was used to determine the homogeneity degree of the irradiation dose.

Experimental methods regarding animal care was approved by the Animal Care Committee of Tarbiat Modares University,Iran. Ingredients, composition, and nutritional value of the diets in starter (1-14 days), grower (15-28 days), and finisher (29-42 days) periods are shown in Table 1. Feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), and mortality was recorded weekly and cumulatively.

Chemical analysis of diet

Chemical composition of the diet was determined by using methods described by AOAC (2000) for dry matter (DM) (934.01), crude protein (CP) (968.06), ether extract (EE) (920.39), crude fiber (CF) (962.09), and ash (942.05).

Microbial assay

Irradiated feed samples (10 g) were placed in 90 mL peptone water (0.1% sterile peptone, w/v) in a sterile stomacher bag.

Serial dilutions (10^{-2} to 10^{-5}) were prepared and total aerobic bacteria (Plate Count Agar; Merck, Darmstadt, Germany) and coliforms (MacConkey Agar; Merck, Darmstadt, Germany) were counted.

Two birds from each replicate were sacrificed by cervical dislocation at 21 and 42 days of age. Meckel's diverticulum to ileocecal junction, and the ileocecal junction to the end of the intestine was considered as ileum and caeca, respectively. Digesta samples were gently removed in sterile sampling tubes and immediately cooled on ice. Serial dilutions (10^{-4} to 10^{-7}) were prepared and microbial populations for total aerobic and coliforms were counted after aerobic incubation at 37 ˚C for 24 hours, and lactobacillus (MRS Agar; Merck, Darmstadt, Germany) for 48 hours (Witkamp, 1963).

Intestinal morphology assay

At 21 and 42 days of age, middle sections (3-4 cm) of duodenum, jejunum, and ileum of two birds from each replicate were incised and prepared for a morphology assay (Iji *et al.* 2001). Tissue processing consisted of serial dehydration with ethanol, cleaning with xylene, and impregnation with wax. The processed samples were then fixed in paraffin wax.

Table 1 Ingredients composition and chemical analysis of the basal diets

Ingredient	1-14 d	15-28 d	29-42 d
Maize (g/kg)	498.2	521.1	470.9
Soybean meal (480 g CP/kg)	410.8	350.3	309.6
Wheat (g/kg)	42.0	80.9	146.8
Soybean oil (g/kg)	11.0	12.9	42.3
Dicalcium phosphate (g/kg)	24.6	22.0	20.6
DL-methionine (980 g/kg)	3.4	2.6	1.6
L-lysine (980 g/kg)	2.3	1.9	0.3
Vitamin permix[a] (g/kg)	2.5	2.5	2.5
Mineral permix[b] (g/kg)	2.5	2.5	2.5
Limestone (g/kg)	-	0.5	-
Salt (g/kg)	2.7	2.8	2.8
Calculated analysis			
ME (MJ/kg)	11.80	12.35	12.74
Crude protein (g/kg)	215.3	188.5	180.1
Ether extract (g/kg)	40.4	50.5	65.7
Calcium (g/kg)	8.8	9.2	9.5
Available P (g/kg)	4.4	4.5	4.7
Lysine (g/kg)	11.8	11.2	10.8
Methionine (g/kg)	3.8	3.7	3.6
Methionine + cystine (g/kg)	9.0	8. 2	7.2
Threonine (g/kg)	9.8	9.1	8.7
Tryptophan (g/kg)	8.4	7.7	7.6

[a] Supplied the following per kilogram of diet: vitamin A: 8000 IU; vitamin D3 (cholecalciferol): 3000 IU; vitamin E (DL-alpha-tocopheryl acetate): 25 IU; menadione: 1.5 mg; vitamin B_{12} (cyanocobalamin): 0.02 mg; Biotin: 0.1 mg; Folacin (folic acid): 1 mg; Niacin (nicotinic acid): 50 mg; Pantothenic acid: 15 mg; Pyridoxine (pyridoxine HCl): 4 mg; Riboflavin: 10 mg; Thiamin (thiamin mononitrate): 3 mg.
[b] Supplied the following per kilogram of diet: copper ($CuSO_4$): 10 mg; Iodine $Ca(IO_3)_2$: 1.0 mg; Iron ($FeSO_4.H_2O$): 80 mg; Manganese ($MnSO_4.H_2O$): 100 mg; Selenium ($NaSeO_3$): 0.15 mg; Zinc ($ZnSO_4.H_2O$) 80 mg and Cobalt ($CoSO_4$) 0.5 mg.

Sections were cut (6µm) from the wax tissue on Leica RM2145 microtome (Leica, Jena, Germany), cleared of wrinkles by floating on warm water (55-60 ˚C), and mounted on 10% poly-L-lysine coated slides.

The slides were stained with haematoxylin and eosin. Villous parameters were determined using a computer-aided light microscopic image analyzer (Motic Images, 2000 1.2, Scion Image, Tokyo, Japan). Both villous height and crypt depth were measured and the villous height: crypt depth rate was determined. Means values of 10 adjacent, vertically oriented villous-crypt units per section were considered for analysis.

Digestibility measurement

Titanium oxide (3 g/kg) was used as an indigestible marker to determine nutrient digestibility (Short *et al.* 1996). After a four-day adaptation period, the ileal digesta were gently removed at 42 days of age (Gong *et al.* 2002). Digesta samples of two birds per replicate were pooled and stored at -20 ˚C until further processing.

Oven-dried samples of feed and digesta were ground (2 mm) and analyzed for chemical composition. Gross energy was determined by adiabatic bomb calorimeter standardized using benzoic acid (Gallenkamp Co Ltd, London, United Kingdom).

The apparent metabolizable energy corrected for nitrogen (AMEn), and coefficients of apparent ileal digestibility (CAID) of DM, CP, organic matter (OM) and EE were calculated according to standard procedure using total collection and marker methods (Kluth and Rodehutscord, 2010). Titanium was determined based on the method described by Short *et al.* (1996). Briefly, the samples were ashed before digestion in 60% sulfuric acid (v/v). The mixture was incubated in 30% H_2O_2, and absorbance was read at 405 nm by an atomic absorption spectrometer (Spectra AA 55B, Varin, USA).

Statistical analysis

A total of four treatments with 4 replicates of 15 birds were used. Results were subjected to regression and variation analyses. One-way analysis of variance was performed using the general linear model procedure of SAS software (SAS, 2004).

The mortality data was calculated to be normal. The least significant differences (L.S.D.) were used to separate means, and P-values of < 0.05 were considered to be significant. Differences among treatments were separated using polynomial orthogonal contrasts to determine linear, quadratic, and cubic responses.

RESULTS AND DISCUSSION

Chemical analysis of diets and microbial assay

Irradiation did not affect the chemical composition of diets (Table 2).

Irradiation at a dose of 5 and 7 kGy eliminated both aerobic bacteria and coliforms (Table 3). Additionally, irradiation decreased (L: P<0.001; Q: P<0.0001) the total aerobic and coliform counts of diets at an increasing rate at 1-14, 15-28, and 29-42 days of age.

No significant difference was observed in the count of total aerobic bacteria in ileum and cecum, and total coliforms in cecum at 21 and 42 days of age (Table 4). Furthermore, no significant differences were observed in total aerobic count in ileum and total coliforms in cecum at 42 days of age.

Total coliforms of the ileum decreased at a declining rate with increasing irradiation dose at 21 (Q: P<0.0001; C: P<0.05) and 42 days of age (Q: P<0.0001).

Lactobacillus counts rose at an increasing rate with increased EBI dose, except for cecum lactobacillus at 42 days of age.

Table 2 Effects of electron-beam irradiation on chemical composition of the diet fed from 15-28 days of age (g/kg)

	Dose of irradiation (kGy)				SEM	P-value	Contrasts		
	0	3	5	7			L	Q	C
Dry matter	874.7	876.6	874.6	871.4	0.196	0.852	NS	NS	NS
Crude protein	188.8	188.4	188.1	187.8	0.186	0.998	NS	NS	NS
Ash	44.8	44.0	44.0	43.7	0.190	0.997	NS	NS	NS
Crude fiber	50.9	50.1	49.4	49.0	0.178	0.987	NS	NS	NS
Ether extract	55.0	54.6	55.6	53.0	0.194	0.977	NS	NS	NS

L: linear contrast; Q: quadratic contrast and C: cubic contrast.
SEM: standard error of the mean and NS: non significant.
Each value represents four replications.

Table 3 The effects of electron-beam irradiation on microbial contamination of the prepared diets (1-14, 15-28 and 29-42 d)

Total aerobial (Log_{10} cfu/g)	Dose of irradiation (kGy)				SEM	P-value	Contrasts		
	0	3	5	7			L	Q	C
14 day	6.46^{x}	4.27^{y}	ND^{c}	ND	0.732	0.0001	***	****	NS
28 day	6.28^{x}	4.77^{y}	ND	ND	0.737	0.0001	***	****	NS
42 day	6.56^{x}	4.49^{y}	ND	ND	0.749	0.0001	****	****	NS
Total coliform (Log_{10} cfu/g)									
14 day	6.28^{x}	4.35^{y}	ND	ND	0.720	0.0001	***	****	NS
28 day	5.82^{x}	4.03^{y}	ND	ND	0.669	0.0001	***	****	NS
42 day	5.35^{x}	3.88^{y}	ND	ND	0.624	0.0001	***	****	NS

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
L: linear contrast; Q: quadratic contrast and C: cubic contrast.
SEM: standard error of the mean.
NS: non significant; *** P<0.001 and **** P<0.0001.
Each value represents four replications (four samples were given for each treatment).

Table 4 Effects of irradiation on intestine microbial population (Log_{10} cfu/g) of broiler at 21 and 42 days of age

Ileum day 21	Dose of irradiation (kGy)				SEM	P-value	Contrasts		
	0	3	5	7			L	Q	C
Total aerobic	9.22	9.15	9.17	9.14	0.015	0.333	NS	NS	NS
Total coliforms	6.51^{x}	6.45^{xy}	6.40^{yz}	6.33^{z}	0.019	0.002	NS	****	*
Lactobacillus	8.99^{y}	9.01^{b}	9.07^{xy}	9.12^{x}	0.020	0.080	NS	**	NS
Cecum day 21									
Total aerobic	8.92	8.91	8.87	8.84	0.021	0.512	NS	NS	NS
Total coliforms	9.04	9.01	8.96	8.94	0.027	0.568	NS	NS	NS
lactobacillus	10.29^{z}	10.33^{yz}	10.40^{xy}	10.44^{x}	0.022	0.032	NS	***	NS
Ileum day 42									
Total aerobic	10.09	10.02	10.04	10.01	0.017	0.408	NS	NS	NS
Total coliforms	7.48^{x}	7.40^{x}	7.19^{y}	7.12^{y}	0.043	0.000	NS	****	NS
Lactobacillus	9.84^{z}	9.92^{yz}	10.01^{xy}	10.09^{x}	0.029	0.001	NS	****	NS
Cecum day 42									
Total aerobic	10.12^{x}	9.78^{y}	9.74^{y}	9.71^{y}	0.045	0.000	****	****	**
Total coliforms	9.88	9.86	9.81	9.79	0.023	0.482	NS	NS	NS
Lactobacillus	11.08	11.12	11.20	11.24	0.039	0.516	NS	NS	NS

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
L: linear contrast; Q: quadratic contrast and C: cubic contrast.
SEM: standard error of the mean.
NS: non significant; * P<0.05; ** P<0.01; *** P<0.001 and **** P<0.0001.
Each value represents eight replications.

Growth performance and apparent ileal digestibility

Feed intake, mortality (all periods), BWG, and FCR were similar between the treatment groups at 1-14 and 15-28 days of age. However, BWG improved at an increasing rate (Q: P<0.001) and FCR decreased (Q: P<0.01) when the irradiation dose increased during 28-42 and 1-42 days of age (Table 5). Diet CAID (coefficients of apparent ileal digestibilities) of DM, OM, EE, AMEn, and gross energy showed a linear increase with radiation dose (Q: P<0.001) (Table 6). No significant difference was observed in CP digestibility.

Intestinal morphology

There was a linear increase in villus height (duodenum, jejunum and ileum) with the enhanced irradiation dose. Additionally, a linear increase and decrease in villus height: crypt depth in the jejunum was observed at 21 and 42 days of age, respectively (P<0.05).

Table 5 Performance of broilers fed electron-beam irradiation diets from 1 to 42 days of age

1-14 day	Dose of irradiation (kGy)				SEM	P-value	Contrasts		
	0	3	5	7			L	Q	C
Feed intake (g/d)	47.6	48.0	47.6	47.4	0.388	0.966	NS	NS	NS
Body weight gain (g/d)	32.7	33.3	33.6	34.4	0.373	0.481	NS	NS	NS
Feed conversion ratio	1.45	1.44	1.42	1.38	0.021	0.662	NS	NS	NS
Mortality (%)	4.77	4.55	4.44	4.22	0.201	0.864	NS	NS	NS
14-28 day									
Feed intake (g/d)	125.3	127.9	126.7	126.9	0.595	0.526	NS	NS	NS
Body weight gain (g/d)	70.0	72.9	74.5	74.9	0.959	0.271	NS	NS	NS
Feed conversion ratio	1.79	1.75	1.70	1.69	0.020	0.295	NS	NS	NS
Mortality (%)	0.68	0.00	0.00	0.00	0.290	0.0601	NS	NS	NS
28-42 day									
Feed intake (g/d)	197.2	195.4	197.8	196.9	1.415	0.956	NS	NS	NS
Body weight gain (g/d)	92.5^{z}	93.6^{yz}	98.5^{xy}	103.8^{a}	1.440	0.005	NS	***	NS
Feed conversion ratio	2.13^{x}	2.08^{x}	2.01^{xy}	1.89^{b}	0.032	0.033	NS	**	NS
Mortality (%)	0.00	0.22	0.00	0.00	0.103	0.062	NS	NS	NS
1-42 day									
Feed intake (g/d)	121.9	122.9	124.9	123.7	0.671	0.478	NS	NS	NS
Body weight gain (g/d)	65.4^{y}	66.6^{y}	68.9^{xy}	71.0^{a}	0.767	0.024	NS	***	NS
Feed conversion ratio	1.77^{x}	1.75^{x}	1.72^{xy}	1.65^{b}	0.017	0.073	NS	*	NS
Mortality (%)	5.45	4.77	4.44	4.22	0.152	0.811	NS	NS	NS

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
L: linear contrast; Q: quadratic contrast and C: cubic contrast.
SEM: standard error of the mean.
NS: non significant; * P<0.05; ** P<0.01 and *** P<0.001.
Each value represents four replications (pen with 15 chicks).

Table 6 Irradiation effects on coefficients of apparent ileal digestibilities of the diets (42 days age)

	Dose of irradiation (kGy)				SEM	P-value	Contrasts		
	0	3	5	7			L	Q	C
Organic matter	0.724^{y}	0.723^{y}	0.762^{xy}	0.784^{x}	1.16	0.033	NS	***	NS
Dry matter	0.671^{y}	0.718^{y}	0.723^{xy}	0.762^{x}	0.99	0.039	NS	***	NS
Crude protein	0.734	0.720	0.744	0.754	1.01	0.721	NS	NS	NS
Ether extract	0.619^{y}	0.623^{y}	0.674^{x}	0.684^{x}	1.04	0.029	NS	***	NS
Gross energy	0.785^{y}	0.801^{y}	0.856^{x}	0.881^{x}	1.26	0.004	NS	****	NS
$AMEn^{c}$ (MJ/kg)	11.800^{y}	11.875^{y}	12.001^{xy}	12.192^{x}	0.05	0.017	NS	***	NS

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
L: linear contrast; Q: quadratic contrast and C: cubic contrast.
SEM: standard error of the mean.
AMEn: apparent metabolizable energy corrected by nitrogen.
NS: non significant; *** P<0.001 and **** P<0.0001.
Each value represents eight replications.

The villus height: crypt depth ratios of duodenum and jejunum followed a linear increasing rate (Q: P<0.001) with increased irradiation doses (Table 7).

Microbial and chemical diet analysis

Increased EBI dose reduced the total microbial population. Louise *et al.* (2006) reported that irradiation by the disruption of key metabolic functions such as energy production, enzymatic activity, or increased energy demand on cell homeostasis could repress DNA repair mechanisms, thereby, arresting and inhibiting growth of microorganisms. Lee *et al.* (1998) reported that EBI reduced microorganism counts and enhanced shelf life of ginseng powder.

The results of current study are in agreement with those of other researchers (Louise *et al.* 2006; Kim *et al.* 2006) and indicated that irradiation reduces the microbial load.

The sensitivity of microorganisms to radiation is known to depend on diet composition (Molins, 2001). Chemical composition of the diets were not affected by EBI, which is in agreement with the study of Farag (1989), who reported that irradiation of soybeans at dose levels of 0 to10 kGy did not affect CP, EE, or ash contents. Moreover, Ismail and Osman (1976) confirmed that irradiation of broad beans (*Vicia faba*) at levels of 2.5, 5, 10, and 20 kGy did not induce any significant changes in diet chemical composition. Furthermore, studies have shown that diet nutrients are more radiation-resistant in their pure states (Diehl and Scherz, 1975).

Growth performance

Electron-beam irradiation did not affect FI. Irradiation may improve nutrient utilization, and thus FCR and BWG (Farag, 1998).

Table 7 Intestinal mucosal morphometry of chicks on the different diets

21 d of age	Dose of irradiation (kGy)				SEM	P-value	Contrasts		
	0	3	5	7			L	Q	C
Villus height, µm									
Duodenum	977^{xy}	968^{y}	1029^{xy}	1044^{x}	13.59	0.113	*	NS	NS
Jejunum	767^{y}	772_{y}	812^{xy}	869^{x}	16.01	0.066	**	NS	NS
Ileum	457^{y}	469^{xy}	518^{xy}	543^{x}	15.15	0.138	*	NS	NS
Crypt depth, µm									
Duodenum	209	200	202	212	9.16	0.974	NS	NS	NS
Jejunum	174	166	161	150	6.06	0.622	NS	NS	NS
Ileum	135	131	130	120	5.01	0.785	NS	NS	NS
Villus height:Crypt depth									
Duodenum	4.88	4.94	5.20	5.12	0.26	0.978	NS	NS	NS
Jejunum	4.44^{y}	4.66^{xy}	5.11^{xy}	6.01^{x}	0.25	0.127	*	NS	NS
Ileum	3.49	3.68	4.04	4.52	0.19	0.297	NS	NS	NS
42 d of age									
Villus height, µm									
Duodenum	1239^{y}	1242^{y}	1272^{xy}	1298^{x}	8.25	0.014	***	NS	NS
Jejunum	1002^{y}	1016^{y}	1071^{xy}	1123^{x}	18.13	0.049	***	NS	NS
Ileum	747^{y}	759^{xy}	808^{xy}	833^{x}	15.15	0.138	*	NS	NS
Crypt depth, µm									
Duodenum	246	245	241	243	1.70	0.709	NS	NS	NS
Jejunum	225	223	201	198	5.65	0.191	*	NS	NS
Ileum	178	173	174	157	4.75	0.470	NS	NS	NS
Villus height:Crypt depth									
Duodenum	5.02^{z}	5.07^{yz}	5.28^{xy}	5.34^{x}	0.04	0.026	***	NS	NS
Jejunum	4.43^{y}	4.55^{y}	5.30^{xy}	5.84^{x}	0.20	0.034	***	NS	NS
Ileum	4.28	4.43	4.71	5.28	0.18	0.248	NS	NS	NS

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
L: linear contrast; Q: quadratic contrast and C: cubic contrast.
SEM: standard error of the mean.
NS: non significant; * P<0.05; ** P<0.01 and *** P<0.001.
Means values represents 10 villous-crypt units for each replicate. Each value represents eight replications.

The results of present study confirmed this finding and agree with the findings of others (Farag, 1998; Zeb *et al.* 2002; Derouchey *et al.* 2003), wherein, irradiation improved FCR and BWG, but had no effect on feed intake. The observations of this study indicated that irradiation could improve the digestibility and nutritional quality of different ingredients by reducing the anti-nutritional factors (Ghazy, 1990). It has previously been established that diet ingredients (maize, soybean meal, and wheat) contain some anti-nutritive factors (Siddhuraju *et al.* 2002; Engberg *et al.* 2004). Improved performance with EBI observed by Campbell *et al.* (1983) is in agreement with the results of the present study. It has also been reported that irradiation splits AA peptide bonds (Elias and Cohen, 1977) and increases the sensitivity of starch to α- and β-amylase (Ananthaswamy *et al.* 1970). Hence, irradiation effects on diet structure and composition seem to be the main reason for improved performance of chickens.

Coefficients of apparent ileal digestibility

The beneficial effects of irradiated feed on broiler performance are likely related to more efficient nutrient use. Irradiated diets may alter the microflora of the gastrointestinal tract and ultimately affect nutrient utilization.

Numerous studies (Farag, 1998; Mani and Chandra, 2003; AL-Masri, 2003) have reported an improvement in the DM, OM, and ash digestibility, as well as AMEn of diets with feed irradiation. Moreover, irradiation has improved the apparent absorption of fat, amino acids, starch (Campbell *et al.* 1983), and net protein utilization in chicks (Nene *et al.* 1975). However, Al-Masri (2003) reported that feeding irradiated meat-bone meal to broilers had no significant effect on energy values, which contradicts with current findings. Irradiation can decompose linkage structures (Sandev and Karaivanov, 1977) and help to facilitate nutrient digestibility (Lacroix *et al.* 1983).

It appears that irradiation results in depolymerization and decomposition of fiber contents (Saeman *et al.* 1952). In fact, digestibility increased in samples treated with irradiation. Therefore, it seems that improvement in nutrient digestibility is caused by modification of the overall chemical structures of diet components.

Microbial population

Microbial populations of the gastrointestinal tract have major influence on intestinal functions (Shakouri *et al.* 2009). In this study, irradiated feed reduced coliform and total aerobic bacteria counts at 21 and 42 days of age.

This is in agreement with the finding of Engberg *et al.* (2002), who reported that feeding birds mash diets induced beneficial microbial populations in the gastrointestinal tract. Thus, diet can be considered as the most important factor affecting the microflora.Nutrient concentration (Williams *et al.* 2001), diet composition and form (Hubener *et al.* 2002), source of dietary energy, and type of diet fat (Knarreborg *et al.* 2002) can modify the gastrointestinal tract conditions affecting the access and amount of substrate, resulting in microbial population changes (Barrow, 1992). Therefore, it seems that diet irradiation affects its structure and can ultimately alter the microbial population. Additionally, irradiation may eliminate microbial agents and toxins in the diet.

Intestinal morphology

Dietary treatment affected intestinal morphology characteristics of broilers in various ways. Irradiation has been shown to reduce anti-nutritional factors in the diet (Siddhuraju *et al.* 2002) and may affect small intestine morphology. An increase in the irradiation dose from 0 to7 kGy hindered villus height suppression, as villus height was increased with higher doses of irradiation. The crypt has an important role in cell proliferation, and its alteration indicates tissue turnover changes and demand for new tissue (Xu *et al.* 2003). Therefore, crypt depth without changes may suggest that villous turnover is not affected by diet (Iji *et al.* 2001). Crypt depth was similar between the treatments in this study, which implies that irradiation of diets had no effects on villous turnover and cell proliferation. Both the highest villus height and villus height: crypt depth of the small intestine was observed with a 7 kGy dose. Additionally, broilers fed diets irradiated with a dose of 7 kGy had the highest BWG. Thus, it may be concluded that irradiated diets can induce favorable intestinal morphology characteristics, leading to increased utilization of feed, and improvement in overall broiler performance.

CONCLUSION

Irradiated diets reduce microbial coliform counts, increased lactic acid producers in the gastrointestinal tract, and improved intestinal villus parameters. The apparent ileal digestibility of all the nutrients and metabolizable energy were improved with irradiation. Improved body weight gain and feed conversion ratio of broilers fed irradiated diets may be explained by irradiation effects on nutrient's digestibility.

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