African Journal of Agricultural Research Vol. 4 (7), pp. 592-598, July 2009
Available online at http://www.academicjournals.org/AJAR
ISSN 1991-637X © 2009 Academic Journals

Full Length Research Paper

The effect of autoclave processing and gamma irradiation on apparent ileal digestibility in broiler breeders of amino acids from canola meal

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Accepted 22 June, 2009

The objective of this study was to investigate the effect of different doses of gamma irradiation and autoclaving on the apparent ileal digestibility in male broiler breeders of amino acids from canola meal. Samples were irradiated in a gamma cell at total doses of 15, 30 and 45 kGy. One package (control) was left at a room temperature: similar to the other treatments, evaporation decreased the moisture content of the samples. Autoclaving of canola meal for 15 min at 121°C was studied. The treatments were: 1) control untreated canola meal diet; 2) autoclaved canola meal diet; 3) canola meal diet gamma irradiated at a dose of 15 kGy; 4) canola meal diet gamma irradiated at a dose of 30 kGy; 5) canola meal diet gamma irradiated at a dose of 45 kGy. The results showed that autoclaving for 15 min at 121°C had a statistically significant effect on the apparent ileal digestibility of the amino acids in canola meal ($p < 0.05$). Autoclaving increased the apparent ileal digestibility of the amino acids of canola meal compared with the other treatments ($p < 0.05$). The effect of autoclaving on an increase in total coefficient of ileal apparent of amino acid digestibility (CIAD) total apparent ileal digestibility of indispensable amino acids, and total apparent ileal digestibility of dispensable amino acids was significant ($p < 0.05$). Gamma irradiation at doses of 15, 30 and 45 kGy significantly decreased the content of glucosinolate of canola meal compared with that of untreated canola meal ($p < 0.05$). The results also showed that gamma irradiation of canola meal were effective in denaturizing of protein and in increasing the apparent digestibility of amino acids. In addition, autoclaving canola meal for 15 min at 121°C and 105 kPa increased the apparent digestibility of amino acids. Irradiation of canola meal at 45 kGy improved the apparent digestibility of amino acids in comparison with the other processing methods.

Key words: Gamma, digestibility, protein, canola, poultry.

INTRODUCTION

Canola meal is a good source of protein for animals and is characterized as having lower and constant amino acid digestibility and metabolizable energy levels than soybean meal (NRC, 1994). However, canola meal contains antinutritional factors that are responsible for low utilization of its nutrients.

These antinutritional factors are: glucosinolates, erosic acid, phytic acid, tannin, NSP (non-starch polysaccharides), sinapine and a high level of fibre. The high level of fibre in canola meal is responsible for its low metabolizable energy (Newkrik et al., 2003).

In addition to these antinutritional factors, the processing conditions affect the quality of canola meal. For example, extensive heating of oilseed meals during processing can lead to a decrease in the content and digestibility of amino acids (Parsons et al., 1992). Removal of undesirable components is essential to improve the nutritio-

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nal quality of meals and to utilize their potential as animal feeds effectively. Several conventional food processing methods, such as germination (Nnanna et al., 1990), soaking (Vidal-Valverde et al., 1994), cooking (Urbano et al., 1995), fermentation (Yamamato et al., 1992) and gamma irradiation (Abu-Tarbonsh, 1998), are known to reduce antinutritional factors effectively and to upgrade the nutritional quality of feeds of plant origin. However, most of these treatments affect the sensory characterristics of the final product adversely. Autoclaving is a heat treatment, and an additional technique is the application of gamma irradiation, which has been used to decontaminate food by killing bacteria, insects, and other foodborne pathogens. Irradiation is also used to increase the shelf-life of fresh and dry food materials (Molisons, 2001). Irradiation of food is a physical process that involves an energy input, but it does not induce radioacti-vity in the food. The amount of energy input is called the radiation absorbed dose, and it is measured in Grays (1 Gy = 1 J/kg). The process is similar in nature to the use of heat via either thermal (infrared) or microwave energy. In contrast to the gross and easily detectable effect that conventional heat treatments have on foods, the radiation dose generates minute and mostly undetectable changes in the chemical composition of the food (Siddhuraju et al., 2002). Irradiation of food has been recognized as a reliable and safe method for preservation of food and of improving the hygienic and nutritional quality of foods (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). The chemical changes that result from the irradiation of proteins in food have been subjected to considerable study (Elias and Cohen, 1997; WHO, 1981).

There is recent evidence that amino acid digestibility in a corn- soybean meal diet is lover during the first 7 days of age than at later ages (Batal and Parsons, 2002). Furthermore, amino acid digestibility coefficients determined by the chick assay for different feed ingredients were lower in 14-d-old chicks than in 28 or 42-d old chickens, possibly indicating that the amino acid digestibility values obtained by the rooster assay might overestimate those for young chicks (Huang et al., 2005). The digestive capabilities of 7-d-old chicks are not fully developed, which could explain the lower amino acid digestibility in birds of that age as compared with adult roosters (Sklan, 2001). Lower amino acid digestibility in young chickens has been well documented. Lower digestibility coefficients for some amino acid obtained in 29-d-old chickens as compared with those obtained at 43 d of age (Ten Doeschate et al., 1993). Increasing amino acid digestibility from 14 to 28 d and in some cases to 42 d of age has been reported for several feed ingredients (Huang et al., 2005).

The objective of this study was to investigate the effect of different doses of gamma irradiation and autoclaving on the apparent ileal digestibility of amino acids in canola meal in male broiler breeders at the age of 7 weeks.

MATERIALS AND METHODS

Gamma irradiation

Sample preparation and irradiation and autoclaving treatments of the canola meal were provided by the Oilseed Development and Cultivation Company (Tehran, Iran). The canola meal used in this study was assayed at 921 g DM/kg. This value was determined by oven drying a 1 g sample in duplicate prior to processing. The moisture content of the canola meal was increased to 250 g/kg with distilled water.

The canola meal samples were divided into four equal portions and placed in paper packages. Three paper packages of samples were irradiated in a gamma cell to total doses of 15, 30 and 45 kGy in the presence of air. One package (control) was left at room temperature: similar to the other samples, evaporation decreased the moisture content of the samples in paper packages. After completing the 45 kGy irradiation and prior to sealing the samples in plastic bags, all samples were spread in trays and allowed to equilibrate with air for 2 h. Gamma irradiation was carried out in the Nuclear Research Center for Agriculture and Medicine of the Iranian Atomic Energy Organization. The irradiation procedure used a gamma cell 220 research irradiator at room temperature. The dose rate, determined by Fricke dosimetry, which was used to calculate the apparent ileal digestibility of amino acids in canola, was 0.36 Gy/s (Holm and Berry, 1970).

Autoclaving

Autoclaving of canola meal was performed for 15 min at 121°C.

Birds and housing

A total of 80 male broiler breeders (5 weeks old; ARBOR ACERS) of uniform body weight (BW; 1.5 to 1.8 kg) were obtained from a commercial farm and allocated to 20 individual pens (four birds per pen).

Four replicate pens were then randomly assigned to each assay diet. The birds were kept in a temperature-controlled building on concrete floor pens. The experimental units (pens) were allocated at random to the five dietary treatments. The birds received a commercial diet in mash form and were allowed to adapt to the pens until the experimental diets were assigned. Feed and water were supplied *ad libitum* and the birds received continuous fluorescent lighting throughout the study.

Assay diets

The canola meal assay diets were based on dextrose

Table 1. Composition of experimental semi-purified diet containing canola meal.

Ingredients (%)	Amount
Canola meal	57
Glucose	38
Dicalcium phosphate	1.3
Oyster shell	1.0
Vegetable oil	1.2
Trace mineral–vitamin premix	0.5
Chromic oxide	0.4
Salt	0.25
Choline chloride	0.2
$NaHCO_3$	0.15
Calculated composition	
ME (kcal/kg)	2735
CP (%)	17

Table 2. Chemical composition and amino acid concentration of canola meal.

Nutrient	Amount
DM (%)	92.1
CP (%)	33.9
Ash (%)	6.1
Ether Extract (EE)	3.3
Crud Fibre (CF)	13.5
Amino acids (%)	
Alanine	1.57
Arginine	2.13
Aspartic acid	2.42
Glutamic acid	6.31
Glycine	1.82
Histidine	1.01
Isoleucine	1.49
Leucine	2.54
Lysine	2.24
Methionine	0.41
Phenylalanine	1.41
Serine	1.16
Threonine	1.41
Tyrosine	1.11
Valine	1.95
Proline	2.15

(glucose) (Table 1). Canola meal was the sole source of dietary protein, and a semi-purified diet was formulated to give the five test diets. The treatments were:

1. Control: untreated canola meal diet.
2. Autoclaved canola meal diet.
3. Canola meal diet gamma irradiated at a dose of 15 kGy.
4. Canola meal diet gamma irradiated at a dose of 30 kGy.
5. Canola meal diet gamma irradiated at a dose of 45 kGy.

An inert external marker, chromic oxide (Cr_2O_3) was used in the treatment diet to estimate feed intake quantitatively. After the acclimatization period, on day 42 the birds were weighed and birds within a narrow weight range were assigned to pens of four birds each. The birds were fasted overnight, and from day 43 - 47 each assay diet was offered ad libitum to four pens (four birds per pen).

Each kg of premix contained the fallowing: trans-retinol, 0.66 mg; cholecalciferol, 0.018 mg; DL-α tocopherol acetate, 4 mg; menadione, 0.4 mg; thiamine, 0.3 mg; riboflavin, 1.6 mg; calcium pantotenate 3 mg; niacin, 0.6 mg; pyridoxine, 1 mg; folic acid, 0.4 mg; cyanocobalamin, 0.3 µg; biotin, 0.02 mg; manganese, 15 mg; zinc, 10 mg; iron, 4 mg; copper, 1 mg; iodine, 0.2 mg; cobalt, 0.06 mg; selenium, 0.02 mg; molybdenum, 0.32 mg; choline chloride, 60 mg; etoxyquin, 25 mg.

Collection of ileal digesta

Digesta from the birds within a pen were pooled and immediately stored at -20°C in airtight containers. After 4 days on the assay diet, the birds were euthanized by intravenous injection inter being anaesthesia. The ileum from Merckel's diverticulum to a point 4 cm proximal to the ileo-junction were gently flushed using sterile distilled water. The body cavity was opened, and the ileum removed. The samples were subsequently freeze-dried and ground to pass through a 0.5 mm sieve (Huang et al., 2006).

Chemical analysis

The amino acid content of the canola meal diet and the samples of ileal digesta were determined by high performance liquid chromatography (HPLC) according to the procedures described by Ravindran et al. (1999). The crude protein, dry matter, crude fibre, ether extract (EE) and ash content of the canola meal are shown in Table 2. They were determined according to the methods of AOAC (1990). Triplicate determinations of the content of chromium oxide (Cr_2O_3) of the diets and the samples of digesta were made using atomic absorption spectrophotometry, following the procedure of Williams et al. (1963). The total content of ileal digesta was determined by intravenous injection of 0.3 ml/kg BW ketamine 10%, immediately, according to the procedure of Mullan et al. (2000).

Calculations and statistical analysis

The apparent amino acid digestibility of the treatment

Table 3. Apparent ileal digestibility of amino acids in canola meal fed to broiler breeders[1, 2].

Nutrients	Untreated	Autoclaved	Irradiation dose (kGy)		
			15	30	45
Indispensable amino acids					
Arginine	82.13[3] ± 0.49 [c]	89.34 ± 0.37 [b]	89.86 ± 0.47 [b]	90.36 ± 0.25 [b]	93.02 ± 0.39 [a]
Lysine	81.64 ± 0.55 [b]	91.70 ± 0.73 [a]	84.04 ± 0.89 [b]	83.92 ± 0.96 [b]	90.31 ± 0.75 [a]
Methionin	91.30 ± 0.58 [bc]	92.64 ± 0.49 [ab]	87.82 ± 0.80 [d]	89.81 ± 0.53 [c]	94.02 ± 0.52 [a]
Histidin	81.46 ± 0.56 [b]	89.30 ± 0.99 [a]	82.54 ± 0.99 [b]	87.49 ± 0.81 [a]	90.07 ± 1.10 [a]
Isoleucine	79.41 ± 1.80 [c]	94.12 ± 0.49 [a]	81.22 ± 1.10 [c]	88.35 ± 0.63 [b]	90.35 ± 1.00 [b]
Leucine	82.23 ± 0.25 [d]	93.08 ± 0.34 [a]	86.99 ± 0.36 [c]	89.25 ± 0.33 [b]	93.28 ± 0.39 [a]
Phenylalanine	82.74 ± 0.76 [c]	90.72 ± 0.76 [a]	84.39 ± 0.70 [bc]	84.59 ± 0.53 [bc]	86.24 ± 0.42 [b]
Valine	81.73 ± 0.48 [bc]	89.87 ± 0.66 [a]	80.93 ± 0.53 [c]	83.14 ± 0.61 [b]	89.33 ± 0.50 [a]
Thereonine	78.92 ± 0.54 [c]	88.17 ± 0.90 [a]	80.28 ± 0.83 [c]	83.73 ± 0.92 [b]	86.89 ± 0.55 [a]
Dispensable amino acids					
Tyrosine	78.86 ± 0.76 [c]	80.12 ± 1.10 [bc]	79.61 ± 0.76 [bc]	82.03 ± 0.99 [b]	91.18 ± 0.82 [a]
Alanine	81.74 ± 0.52 [d]	89.29 ± 0.75 [a]	84.85 ± 0.47 [c]	86.13 ± 0.47 [bc]	87.39 ± 0.41 [b]
Proline	83.05 ± 0.28 [c]	91.41 ± 0.53 [a]	83.93 ± 0.45 [c]	85.65 ± 0.54 [b]	92.71 ± 0.37 [a]
Aspartic acid	82.54 ± 0.46 [e]	94.13 ± 0.48 [b]	85.27 ± 0.64 [d]	91.94 ± 0.72 [c]	97.19 ± 0.22 [a]
Glutamic acid	78.89 ± 1.40 [c]	88.02 ± 1.10 [ab]	79.80 ± 0.93 [c]	84.89 ± 1.20 [b]	89.21 ± 0.64 [a]
Glycine	82.48 ± 0.50 [e]	91.29 ± 0.50 [b]	84.30 ± 0.78 [d]	87.72 ± 0.43 [c]	93.16 ± 0.53 [a]
Serine	78.79 ± 0.52 [c]	82.42 ± 0.82 [b]	78.17 ± 0.90 [c]	82.34 ± 0.81 [b]	85.20 ± 0.80 [a]
Total*	81.75 ± 0.17 [e]	89.73 ± 0.44 [b]	83.37 ± 0.40 [d]	86.33 ± 0.49 [c]	90.60 ± 0.54 [a]
Total. Dis*	80.91 ± 0.29 [e]	88.10 ± 0.69 [b]	82.28 ± 0.49 [d]	85.81 ± 0.62 [c]	90.86 ± 0.19 [a]
Total. in dis*	82.40 ± 0.14 [d]	90.99 ± 0.32 [a]	84.23 ± 0.36 [c]	86.74 ± 0.52 [b]	90.39 ± 0.82 [a]

* Total amino acid digestibility.
** Total apparent ileal digestibility of indispensable amino acids.
*** Total apparent ileal digestibility of dispensable amino acids.
1. At 6 weeks, a, b, c, d means in the same row with different letters differ ($p < 0.05$).
2. The values are means of four replicate pens (four birds/replication).
3. Mean ± SEM

diets was calculated using the equation below (Short et al., 1999). All statistical analysis was computed using SPSS version 16. The data were analysed as a completely randomized design (Steel and Torrie, 1980) using the General Linear Model (GLM) procedure. The means were compared between individual treatments using Duncan's multiple range test (DNMRT).

$$\text{Coefficient of Ileal apparent AA digestibility (CIAD)}_a = \frac{\text{AA infected}}{\text{marker infeed}} - \frac{\text{AA in ileum}}{\text{marker in ileum}} \Big/ \frac{\text{AA infeed}}{\text{marker in feed}}$$

RESULTS

The results (Table 3) showed that the influence of autoclaving for 15 min at 121°C altered the apparent ileal digestibility of the amino acids of canola meal ($p < 0.05$). Autoclaving increased the apparent ileal digestability of the amino acids in canola meal compared with the other treatments ($p < 0.05$). The effect of autoclaving was to increase total coefficientof ileal apparent amino acid digestibility (CIAD), total apparent ileal digestibility of indispensable amino acids, and total apparent ileal digestibility of dispensable amino acids ($p < 0.05$). The effect of autoclaving on the increase in CIAD of individual amino acids was similar ($p < 0.05$), except for methionine and tyrosine ($p > 0.05$). The results (Table 3) showed that gamma irradiation at doses of 15, 30 or 45 kGy significantly increased the CIAD of canola meal ($p < 0.05$). The increase in CIAD was greatest at a dose of 45 kGy ($p < 0.05$). A dose of 45 kGy significantly increased the CIAD of individual amino acids except isoleucine, phenylalanine, and alanine; for these amino acids the effect of 45 kGy was similar to that of 30 kGy ($P \geq 0.05$). Irradiation with a dose of 30 kGy significantly increased ($p < 0.05$) the CIAD of all individual amino acids expect methionine, phenylalanine, aspartic acid, and alanine compared with the control treatment.

Irradiation with a dose of 15 kGy significantly increased the CIAD of arginine, leucine, alanine, aspartic acid and glycine ($p < 0.05$). Gamma irradiation of canola meal with a dose of 15, 30 or 45 kGy significantly increased the CIAD of the total indispensable and dispensable amino

Table 4. Chemical composition and anti-nutritional factors of untreated and irradiated canola meal (n = 3).

Parameters	Untreated canola meal	Gamma-irradiated canola meal			SEM
		15 KGY	30 KGY	45 KGY	
Glucosinolate (µmol/g)	18.6[a]	13.7[b]	10.3[c]	7.8[c]	0.9

* On dry matter basis
[a, b, c, d] Means in the same row with different letters differ ($p < 0.05$).

acids ($p < 0.05$). Gamma irradiation of canola meal at 15, 30 or 45 kGy significantly increase the CIAD of the amino acids but the greatest effect was seen at a dose of 45 kGy ($p < 0.05$). However, irradiation with a dose of 15 kGy significantly decreased the CIAD of methionine ($p < 0.05$).

Table 4 shows that gamma irradiation at a dose of 15, 30 or 45 kGy significantly decreased the level of glucosenolate ($p < 0.05$). The effect of gamma irradiation at doses of 30 and 45 kGy was similar ($p < 0.05$).

Gamma irradiation at a dose of 15, 30 or 45 kGy significantly decrease the level of glucosinolate in canola meal in comparison with untreated canola meal ($p < 0.05$). Irradiation with a dose of 45 kGy produced the greatest decrease in glucosinolate concentration ($p < 0.05$).

DISCUSSION

There is particular interest in the irradiation of proteins using ionizing radiation for sterilization. Gamma irradiation of proteins may induce structural changes and alter their functional properties. Protein molecules irradiated in the solid state absorb radiation energy directly, producing changes as a result of the so-called “direct effect”. In aqueous solution, radiation acts first on the water molecules, producing active species such as hydroxyl radicals (°OH) and hydrated electrons (e aq) that in turn react with the protein molecules. In this case, the radiolysis of proteins takes place as a result of the “indirect” effects (Yamamoto, 1992). The effects of heat and pressure are closely related to the steps that occur during oil seed extraction, such as pre-heating, cooking, processing, and desolventization (Eskin et al., 1996; Shahidi, 1990; Hamilton et al., 1987). During these steps, heat and pressure are normally required. In a study on sunflower seed phenolics, heating was found to decrease the content of simple phenolics (Sastry and Subramanian., 1985).

Heat treatments were also shown that content of sinapine bisulfate and lignine content of rapeseed were decreased (Jensen et al., 1990). Heat and pressure seem to have a significant effect on the colour and structure of phenolics. However, the exact nature of these changes has rarely been reported, partly because of the complexity of these systems. In addition, there appear to be no reports on the coloration of canola phenolics during autoclaving.

Heat treatment of proteins with steam and under atmospheric pressure resulted in losses of lysine only when the heating time was longer than 40 min. During heat treatment the e-amino group of the lysine may react with reducing sugars (the Millard reaction). Alternatively, dehydroalanine (a decomposition product of cystine or serine) may react with the free amino group of lysine to form lysino-alanine. A third possible reaction is that between the amide group of glutamine and the free amino acid group of lysine, to form a peptide-type linkage with the release of ammonia. Any one or all of these reactions could cause significant loss of available lysine (Lin and Lakin, 1990). According to the definition of the Canola Council of Canada, canola varieties are required to contain less than 30 µmol/g of one or any combination of four known aliphatic glucosinolates, that is, gluconapin, goitrin, gluco-brassicanapin, and napolei ferinin, in the defatted meal (Shahidi et al., 1990). The observed improvement in the biological performance of chicks due to feeding on autoclaved rape seed meal could be related to the reduction in glucosinolates (Aronen and Vanhatalo., 1992), or in soluble tannins and sinapine (Fenwick et al., 1984), as a result of the moist heat treatment.

Generally four types of radiation effects on protein are observed: fragmentation, cross-linkage, aggregation and oxidation by oxygen radicals that are generated in the radiolysis water (Cheftel et al., 1985; Filali-Mouhim et al., 2000).

The hydroxyl and superoxide anion radicals that are generated by radiation could modify the molecular properties of the proteins which result alteration of proteins by covalent cross-linkages formed in proteins after irradiation. Proteins can be converted to higher molecular weight aggregates due to the generation of inter-protein cross-linking reactions, hydrophobic and electrostatic interactions, as well as the formation of disulfide bonds. The cross-linking process results in the formation of Chemical bonds between two adjacent protein molecules (Garrison, 1987).

Protein-Protein interaction increases because the electrostatic forces of molecules are at the minimum and less water interactions with the protein. This is favorable condition for the protein molecules to approach each other and possibly precipitate (Davis and Delsignore, 1987). Moreover, cross-linked proteins are hydrophobic, therefore are compact and could pass to the intestine (Ressouany et al., 1998).

Results in the present work are consistent as mentioned above the chemical changes caused by irradiation in protein include disruption of the ordered structure of protein molecules as well as degradation, cross-linking, and aggregation of the polypeptide chains due to oxygen radicals (Gaber, 2005). Gamma irradiation below 16 KGY was not effective in formation of high molecular weight aggregates in proteins. Therefore gamma irradiation at 15 KGY caused slight breakdown of polypeptide chains (Lee et al., 2005).

In a recent study, it was reported that, when irradiated with doses up to 50 kGy, native ß-LG in solution (sodium phosphate buffer, 10 mM, pH 7.0) undergoes structural alterations and, even though some of the compact structure is maintained, these alterations lead to an ordered aggregation, at least in part mediated by bityrosyl cross-links (Olivera et al., 2007). On the other hand, Olivera et al. (2007) verified that, when the protein was irradiated in the solid state (at different degrees of hydration, and at doses up to 50 kGy), the average size and compactness of the ß-LG molecule did not change. These observations indicate that the conformation of the ß-LG dimer was not affected by radiation (Olivera et al., 2007). To extend the understanding of the relevance of conformational changes at the level of the secondary and tertiary structures that are involved in the aggregation pathway induced by gamma irradiation, samples of ß-LG irradiated in solution (3 or 10 mg ml^{-1}) and in the solid state (with water activities of 0.22, 0.53 or 0.74) were analysed using fluorescence and circular dichroism techniques.

Conclusion

The results of this study show that gamma irradiation of canola meal was effective in denaturizing of proteins and increasing the apparent digestibility of amino acids. In addition, the results indicated that autoclaving canola meal for 15 min at 121°C and 105 kPa increased the apparent digestibility of amino acids. Irradiation of canola meal at 45 kGy improved the apparent digestibility of the amino acids in comparison with the other processing methods studied.

ACKNOWLEDGMENTS

This manuscript is obtained from PhD thesis of Mohammad Molaei at Islamic Azad University, Science and Research Branch, Tehran, Iran. We would like to express thanks for financial support provided by the Islamic Azad University, Science and Research Branch, Tehran, Iran. Moreover the authors gratefully acknowledge Dr. Alireza Seidavi from the Islamic Azad University, Rasht Branch and Mr. R. Alijanpoor and Dr. N. Karimi

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