

Full Length Research Paper

Effect of autoclave processing and gamma irradiation on apparent ileal amino acids digestibility of cottonseed meal in male broiler breeders

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The objective of this study was to investigate the effect of autoclaving and different doses of gamma irradiation on the apparent ileal digestibility of amino acids of cottonseed meal in male broiler breeders. Samples were irradiated in a gamma cell at total doses of 15, 30 and 45 kGy. One package (control) was left at room temperature: Similar to the other treatments, evaporation decreased the moisture content of the samples. Autoclaving of cottonseed meal for 15 min at 121 °C was studied. The treatments were: (1) control, untreated cottonseed meal diet; (2) autoclaved cottonseed meal diet; (3) cottonseed meal diet gamma irradiated at a dose of 15 kGy; (4) cottonseed meal diet gamma irradiated at a dose of 30 kGy; (5) cottonseed meal diet gamma irradiated at a dose of 45 kGy. The results show that autoclaving for 15 min at 121 °C did not have a statistically significant effect on the apparent ileal digestibility of the amino acids in cottonseed meal when compared with the control treatment. The results also show that gamma irradiation of cottonseed meal were not effective in increasing the apparent digestibility of amino acids. In addition, irradiation of cottonseed meal did not improve the apparent digestibility of amino acids in comparison with the other processing methods only, but also significantly decreased it ($p < 0.05$).

Key words: Gamma irradiation, digestibility, amino acid, cottonseed meal, broiler.

INTRODUCTION

Cottonseed meal is not a good source of protein for birds and is characterized by having lower constant amino acid digestibility and metabolizable energy level than soybean meal (NRC, 1994). Cottonseed meal has some anti-nutritional factors that are responsible for low utilization of its nutrients (Newkirk et al., 2003). In addition to these anti-nutritional factors, the processing conditions affect its quality. For example, extensive heating of oilseed meals during processing can lead to loss in the content and digestibility of amino acids (Parsons et al., 1992). Removal of undesirable component is essential to improve the nutritional quality of meals and effectively utilize their potential as poultry feed. Several conventional food processing methods, such as germination

(Nnanna and Philips, 1990), soaking (Vidal-Valverde et al., 1994), cooking (Urbano et al., 1995), fermentation (Yamamoto et al., 1992) and gamma irradiation (Abu-Tarboush, 1998), are known to reduce antinutritional factors effectively and upgrade the nutritional quality of plant origin feeds. However, most of this treatment adversely affects the sensory characteristics of the final product. Autoclaving is a heat treatment, and an additional technique is the application of gamma irradiation, which has already been used to decontaminate food by killing bacteria, insects and other food borne pathogens and also to increase the shelf-life of fresh and dry food materials (Molisons, 2001). Food irradiation is a physical process involving an energy input, but it does not induce radioactivity in foods. The amount of energy input is called the radiation absorbed dose, and measured in Grays (1GY = 1 J/kg). It is similar in nature to the use of heat via either thermal (infrared or

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microwave energies. In contrast to the gross and easily detectable effect that conventional heat treatments have on foods, the radiation dose generates minute and mostly undetectable change in chemical compositions (Siddhuraju et al., 2002). Food irradiation has been recognized as a reliable and safe method for preservation of food which improves the hygienic and nutritional quality of foods (Diehl, 2002). In 1981, the US Food and Drug Administration (FDA) concluded that food irradiation at 50 kGy or less can be considered safe for human consumption (FDA, 1981).

The chemical changes resulting from the irradiation of proteins food have been subjected to considerable study (Elias and Cohen, 1997; WHO, 1981). Most of the published data on digestible amino acids in feed ingredients have been obtained from excreta assays with roosters (NRC, 1994; Green et al., 1997). Although, evidence suggests that ileal digestibility values are better indicator of amino acids availability than excreta-based values (Ravindran et al., 1999),

It is generally assumed that nutrient digestibility does not change between the class of birds, and amino acid digestibility is used in feed formulations of broiler and layers. The applicability of digestibility values generated with adult roosters to chickens is unclear (Bryden and Li, 2003). The objective of this study was to investigate the effect of different doses of gamma irradiation and autoclaving on apparent ileal amino acid digestibility of cottonseed meal in male broiler breeders at age of 7 weeks.

MATERIALS AND METHODS

Gamma irradiation

Sample preparation, irradiation and autoclaving treatments of the cottonseed meal were provided by the Oil-seed Development and Cultivation Company (Tehran, Iran). The cottonseed 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 cottonseed meal was increased with distilled water to 250 g/kg. Then, these cottonseed meals were divided into four equal portions and placed in paper packages. Three paper packages of samples were irradiated in a gamma cell for total doses of 15, 30 and 45 kGy in the presence of air. One package (control) was left at a room temperature similar to the other samples, evaporation decreased moisture content of samples in paper packages. After completing the 45 kGy irradiation and prior to sealing of samples in plastic bags, all samples were spread in trays and allowed to air equilibrate for 2 h. Gamma irradiation was carried out in the Nuclear Research Center for Agricultural 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 amino acid digestibility of cottonseed meal, was 0.36 Gy/s (Holm and Berry, 1970).

Autoclaving

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

Birds and housing

A total of 80 male broiler breeder (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 floor pens (4 birds per pen). Four replicate pens were then randomly assigned to each assay diet. Birds were kept in temperature controlled building on concrete floor pens. The experiment units (pens) were allocated at random to five dietary treatments. The birds received a commercial diet in mash form and were allowed to adapt to the floor 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 cottonseed meal assay diets were based on dextrose (glucose) (Table 1). Cottonseed meal is the sole source of dietary protein, and a semi-purified based diet was formulated to give the five test diets. The treatments were:

1. Control: untreated cottonseed meal diet.
2. Autoclaved cottonseed meal diet.
3. Cottonseed meal gamma irradiated with dose of 15 kGy diet.
4. Cottonseed meal gamma irradiated with dose of 30 kGy diet.
5. Cottonseed meal gamma irradiated with dose of 45 kGy diet.

An inert external marker, chromic oxide (Cr_2O_3) has been used in treatment diet to estimate quantitatively, feed intake. 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 days 43 to 47, each assay diet was given *ad libitum* to birds in four pens (four birds per pen).

Collection of ileal digesta

Digesta from 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 after being anaesthetized. The ileum from Meckel's diverticulum to a point 4 cm proximal to the ileo-cecal junction were gently flushed using sterile distilled water. The body cavity was opened, and the ileum was 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 cottonseed meal diet and ileal digesta samples 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 and ash content of the cottonseed 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 diets and the digesta sample 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 diets was

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

Ingredients(%)	Amount
Cottonseed meal	64
Glucose	27
Dicalcium phosphate	1.4
Oyster shell	1.3
Vegetable oil	5
Vitamin-mineral premix ¹	0.5
Chromic oxide	0.4
Salt	0.3
NaHCO ₃	0.1
Calculated composition	
ME (kcal/kg)	2950
CP (%)	19

¹Each kg of premix contained the following: Trance-retinol, 0.66 mg; cholecalciferol, 0.018 mg; DL- α tocopherol acetate, 4 mg; menadione, 0.4 mg; thiamin, 0.3 mg; riboflavin, 1.6 mg; calcium pantotenat 3 mg; niacin, 0.6 mg; pyridoxin, 1 mg; folic acid, 0.4 mg; cyanocobalamin, 0.3 μ g; 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; molybdenium, 0.32 mg; choline chloride, 60 mg; etoxyquin, 25 mg.

calculated using the equation:

Coefficient of ileal apparent AA digestibility (CIAD) = {(AA in feed/marker in feed) - (AA in ileum/marker in ileum)} / (AA in feed/marker in feed)

All statistical analysis was computed by using SPSS version 16. The data were analyzed 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 rang test (DNMRT).

RESULTS

The results (Table 3) show that the influence of auto-claving for 15 min at 121°C were not statistically significant on apparent ileal digestibility of the amino acids of cottonseed meal as compared with the control treatment. Effect of autoclaving on total coefficient of ileal apparent amino acid digestibility (CIAD), total apparent ileal digestibility of indispensable amino acids, and total apparent ileal digestibility of dispensable amino acids was not significant ($p < 0.05$). Effect of autoclaving on CIAD of individual amino acids was also similar ($p < 0.05$).

The results in Table 3 show that gamma irradiation at doses of 15, 30 or 45 kGy significantly decrease the total coefficient of ileal apparent amino acid digestibility (CIAD), and total apparent ileal digestibility of indispensable amino acids of cottonseed meal ($p < 0.05$). But gamma irradiation at doses of 15, 30 or 45 kGy were not statistically

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

Nutrient (%)	Amount
DM	90.8
CP	30.9
Ash	7.0
Ether extract	2.1
Crude fibre	11.3
Amino acids (%)	
Alanine	0.97
Arginine	2.62
Aspartic acid	2.30
Glutamic acid	6.30
Glycine	1.21
Histidine	0.45
Isoleucine	0.87
Leucine	1.53
Lysine	1.01
Methionine	0.10
Phenylalanine	1.33
Serine	1.10
Threonine	0.94
Tyrosine	0.64
Valine	1.27
Proline	1.46

significant on total apparent ileal digestibility of dispensable amino acids of cottonseed meal ($p < 0.05$). At doses of 15, 30 or 45 kGy on CIAD, valine, threonine, tyrosine, serine and aspartic acid of cottonseed meal was not significant ($p < 0.05$). Also, dose of 15 kGy significantly decreased the CIAD of individual amino acids, except for methionine and glutamic acid as compared to the control group ($p < 0.05$).

DISCUSSION

Heat treatment of protein with steam and under atmospheric pressure resulted in lysine losses only when the heating time was longer than 40 min. During heat treatment, ϵ -amino group of the lysine may react with reducing sugars (the Maillard 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).

There is particular interest in the irradiation of proteins

Table 3. Apparent ileal digestibility of amino acids in cottonseed meal fed to broilers^{1,2}.

Nutrient	Untreated	Autoclaved	Irradiation dose (kGy)			SEM
			15	30	45	
Indispensable amino acids						
Arginine	90.35 ^a	92.94 ^a	83.80 ^{ab}	74.86 ^{ab}	60.68 ^b	7.74
Lysine	94.21 ^a	93.60 ^a	88.42 ^{ab}	72.37 ^b	85.79 ^{ab}	6.43
Methionine	83.82 ^a	88.53 ^a	82.89 ^a	46.53 ^b	57.53 ^{ab}	9.99
Histidine	92.51 ^a	92.17 ^{ab}	90.99 ^{ab}	78.58 ^{ab}	77.62 ^b	4.47
Isoleucine	88.96 ^a	89.38 ^a	81.79 ^{ab}	59.90 ^b	65.64 ^{ab}	8.18
Leucine	90.16 ^a	90.98 ^a	81.07 ^{ab}	60.77 ^b	70.25 ^{ab}	7.98
Phenylalanine	94.18 ^a	94.72 ^a	90.90 ^{ab}	78.25 ^b	81.35 ^{ab}	4.75
Valine	89.88	90.50	83.18	69.49	70.92	6.79
Threonine	91.41	91.94	93.19	81.55	78.14	6.22
Dispensable amino acids						
Tyrosine	91.88	93.77	84.42	85.04	76.60	5.83
Alanine	89.14 ^a	90.06 ^a	79.18 ^{ab}	54.04 ^b	63.00 ^{ab}	10.14
Proline	93.46 ^a	94.17 ^a	89.72 ^{ab}	80.64 ^b	82.83 ^{ab}	3.74
Aspartic acid	98.44	98.52	91.70	91.21	92.79	2.53
Glutamic acid	97.04 ^a	98.51 ^a	95.48 ^a	88.07 ^b	92.16 ^{ab}	2.3
Glycine	94.28 ^a	94.67 ^a	92.04 ^{ab}	81.15 ^b	83.29 ^{ab}	3.95
Serine	94.88	95.16	91.78	82.92	86.87	4.49
Total*	92.16 ^a	93.10 ^a	87.53 ^{ab}	74.08 ^b	76.59 ^{ab}	5.38
Total Dis**	94.16	94.97	89.19	80.44	82.50	4.55
Total Indis***	90.61 ^a	91.64 ^a	86.25 ^{ab}	69.15 ^b	71.99 ^{ab}	6.09

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

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 ($^{\circ}\text{OH}$) and hydrated electrons (e_{aq}) that in turn react with the protein molecules.

In this case, the radiolysis of protein takes place as a result of the “indirect effects” (Yamamoto, 1992). The effects of heat and pressure are closely related to the steps during oil seed extraction, such as pre-heating, cooking, processing and desolventization (Eskin et al., 1996; Shahidi, 1990; Hamilton and Bahail, 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).

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 super oxide anion radicals that are generated by radiation could modify the molecular properties of the proteins which result to 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 a favorable condition for the protein molecules to approach each other and possibly precipitate (Davis and Delsignore, 1987).

Results in this study are consistent with the chemical changes caused by irradiation in protein which 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 the 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 β -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 partly 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 β -LG molecule did not change. These observations indicate that the conformation of the β -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 β -LG-1 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 analyzed using fluorescence and circular dichroism techniques.

Conclusion

The results of this study show that gamma irradiation of cottonseed meal was effective in denaturing of proteins and decreasing apparent digestibility of amino acids. In addition, the results indicate that autoclaving cottonseed meal for 15 min at 121 °C and 105 kPa had no significant effect on apparent digestibility of amino acids.

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