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Ruminal Degradation and *in vitro* Crude Protein Digestibility of Gamma Irradiated Meat and Bone Meal

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Abstract: This study was carried out to determine effects of gamma irradiation (γ-irradiation) at doses of 15, 30 and 45 kGy on ruminal degradability, *in vitro* Crude Protein (CP) digestibility and chemical composition of Meat and Bone Meal (MBM). Nylon bags of untreated or treated MBM were suspended in the rumen of three bulls for up to 48 h and resulting data were fitted to non-linear degradation model to calculate Effective Degradability (ED). Chemical composition of MBM was not affected by γ-irradiation. γ-irradiation decreased ($p<0.05$) the washout fraction and increased ($p<0.05$) the potentially degradable fraction of DM. ED of DM decreased ($p<0.05$) with increases in irradiation dose. γ-irradiation decreased ($p<0.05$) the washout fraction of CP from 252-191 g kg^{-1}. γ-irradiation reduced ($p<0.05$) the degradation rate of the b fraction of CP. γ-irradiation at doses of 15, 30 and 45 kGy decreased ED of CP at rumen outflow rate of 0.05 h^{-1} by 4, 9 and 16%, respectively. γ-irradiation increased ($p<0.05$) *in vitro* CP digestibility of MBM. γ-irradiation at doses of 30 and 45 kGy, increased *in vitro* CP digestibility of undegraded CP remaining in bags incubated for 16 h in the rumen by 13 and 15%, respectively. γ-irradiation seems to be effective in increasing *in vitro* CP digestibility of MBM at doses >15 kGy.

Key words: Meat and bone meal, γ-irradiation, digestibility, degradation kinetics, Iran

INTRODUCTION

Recycling of animal wastes is very useful for economical and environmental aspects. Meat and Bone Meal (MBM) has been used in ruminant nutrition as an escape protein source in Iran. One of the problems arising from the use of recycled animal wastes as MBM in ruminant feed is their susceptibility of being carriers of pathogenic organisms such as *Salmonellae* and *Escherichia coli* to animals (Al-Masri, 2003). Moreover, the CP digestibility of MBM is rather low (NRC, 2001). Heat or irradiation treatments have been used to decrease the pathogen contents of animal wastes (Messer *et al*., 1971; Smith, 1983).

γ-irradiation represented a reliable and safe method for sterilization and preparation of animal feed and by product (Ford, 1976; Ito and Iizuka, 1979; Diehl, 2002). A positive effect of γ-irradiation (25-35 kGy) for sterilization was observed on animal feed (Ito and Iizuka, 1979). Unlike heat treatments, the use of irradiation in the processing of animal feed not only increased CP digestibility but also did not induce significant changes in quality or amino acid composition of their proteins (Caswell *et al*., 1975; Farag, 1998b; Shawrang *et al*., 2007; Ebrahimi *et al*., 2009). As far as we know, no degradability and digestibility data exist in the literature about γ-irradiated MBM.

Therefore, objective of this study was to evaluate effects of γ-irradiation on chemical composition, DM and CP degradability parameters and *in vitro* CP digestibility of MBM.

MATERIALS AND METHODS

Samples preparation and irradiation treatments: The MBM sample was obtained from local commercial market (Tehran, Iran). Samples were mixed and packed in paper packages, then sealed and irradiated at doses of 15, 30 and 45 kGy by using a cobalt-60 irradiator at 20°C. γ-irradiation was completed in the Radiation Application Research School of Atomic Energy Organization of Iran. The dose rate determined by Fricke dosimetry (Holm and Berry, 1970) was 0.325 Gy sec^{-1}. After irradiation and prior to sealing the plastic bags, samples were allowed to air equilibrate for 2 h, then frozen at -18°C.

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Chemical analyses: The DM content was determined in feed samples and nylon bag residues at 55°C for 48 h. The N in feeds and residues after rumen and *in vitro* incubation was determined according to AOAC (1995). Ash was determined by burning duplicate 2 g samples at 600°C for 2 h in a muffle furnace (AOAC, 1995). A standard method was used to determine ether extract (AOAC, 1995).

Animals and diet: Three bulls with an average live weight of 416 kg were fed 8 kg of Dry Matter (DM) of a total mixed ration containing 700 g kg^{-1} of DM forage (700 g kg^{-1} alfalfa hay and 300 g kg^{-1} wheat straw on DM basis) and 300 g kg^{-1} of DM concentrate. The concentrate consisted of ground barley grain, corn grain, canola meal, MBM, wheat bran, dicalcium phosphate and a vitamin + mineral premix (370, 250, 160, 50, 140, 10 and 10 g kg^{-1} DM, respectively). Water and minerals were available *ad libitum*. The diet was formulated according to beef National Research Council (NRC, 1996) guidelines to contain 154 g CP kg^{-1} of DM and was fed twice daily at 08:00 and 16:00 h.

***In situ* ruminal degradability:** Nylon bags (10×20 cm; 45 μm pore size) were filled with approximately 5 g sample (size: bag surface area of 12.5 mg cm^{-2}) according to Michalet-Doreau and Ould-Bah (1992). Duplicate bags were incubated in the rumen for 0, 2, 4, 8, 16, 24 and 48 h. All bags were simultaneously placed in the rumen just before the bulls were offered their first meal (i.e., 08:00 h). After retrieval from the rumen, bags were thoroughly washed with tap water until the rinsing water was clear. The same procedure was applied to two bags to obtain the 0 h value. The residues were dried and analyzed for DM and CP to determine degradation kinetics of MBM.

***In vitro* crude protein digestibility:** Digestibility of rumen undegraded CP was estimated using the three-step *in vitro* procedure of Calsamiglia and Stern (1995). Samples of the ruminal undegradable fraction collected at the 16 h ruminal incubation period containing 15 mg N were incubated for 1 h in 10 mL of 0.1 N HCl solution containing 1 g L^{-1} of pepsin.

Following incubation, the pH was neutralized with 0.5 mL of 1 N NaOH and 13.5 mL of phosphate buffer (pH 7.8) containing 37.5 mg of pancreatin were added. Samples were incubated for 24 h at 38°C and then undigested protein precipitated using trichloroacetic acid (3 mL TCA). Samples were centrifuged 10,000×g for 15 min at room temperature and then supernatant was analyzed for soluble N (AOAC, 1995). *In vitro* digestibility of CP was calculated as soluble N divided by amount of initial sample N (i.e., nylon bag residues).

Statistical analyses: Digestion kinetics of DM or CP was determined according to the equation of Orskov and McDonald as:

$$P = a + b (1 - e^{-ct})$$

Where:

P = The amount degraded at a time
a = The washout fraction
b = The potentially degradable fraction
c = The constant rate of disappearance of b
t = The time of incubation (h)

Effective Degradability (ED) was calculated as:

$$ED = a + [b \times c/(c + k)]$$

Where estimated ruminal outflow rates (k) of 0.02, 0.05 and 0.08 h^{-1} were used.

Degradability data were analyzed as a completely randomized block design according to the general linear models procedure of SAS Institute Inc. (1996) with the statistical model of:

$$Y_{ijk} = \mu + T_i + B_j + e_{ijk}$$

Chemical composition data were analyzed as a completely randomized design according to the GLM procedure of SAS Institute Inc. (1996) with the statistical model of:

$$Y_{ijk} = \mu + T_i + e_{ijk}$$

Where:

Y_{ijk} = Dependent variable
μ = Overall mean
T_i = γ-irradiation effect
B_j = Animal effect
e_{ijk} = Residual error assumed normally and independently distributed. Means were compared by least squares means

RESULTS AND DISCSSION

Effects of γ-irradiation on chemical composition of MBM: The chemical composition of MBM is shown in Table 1. γ-irradiation had no effect on chemical composition of MBM is consistent with results of the other studies (Farag, 1998a; Al-Masri, 2005; Shawrang *et al*., 2007, 2008).

Table 1: Effects of γ-irradiation on chemical composition of Meat and Bone Meal (MBM)

Chemical composition	Untreated MBM	γ-irradiated MBM (kGy)			
		15	30	45	SEM
Dry matter (g kg^{-1})	953.0	948.0	953.0	955.0	4.7.0
Crude protein (g kg^{-1})	533.0	536.0	534.0	534.0	2.4.0
Ether extract (g kg^{-1})	100.0	102.0	99.0	101.0	2.5.0
Ash (g kg^{-1})	304.8	299.8	299.5	301.7	2.66

Effects of γ-irradiation on DM and CP degradability: γ-irradiation decreased ($p<0.05$) the washout fraction (Table 2) and increased ($p<0.05$) the potentially degradable fraction of DM. Effective Degradability (ED) of DM decreased ($p<0.05$) with increased irradiation dose. The washout fraction of CP decreased ($p<0.05$) and increased the potentially degradable fraction of CP ($p<0.05$) with increased γ-irradiation. ED of CP decreased ($p<0.05$) with increased irradiation dose. Maximum potential degradability (a + b) of DM and CP were 655 and 690 g kg^{-1} for untreated MBM, respectively, indicating MBM to be low degradable in the rumen. MBM protein is rich in hydrophobic amino acid such as leucine, isolecine, phenylalanine and methionine, therefore, rumen degradability of it is low. This suggestion is consistent with Prestlokken (1999) and Nasri *et al.* (2008) who reported that hydrophobic amino acids such as leucine, isolecine, phenylalanine, methionine, valine, alanine and tyrosine were degraded to a lesser extent than hydrophilic amino acids as histidine, arginine, lysine, cysteine, glutamic acid and serine. The CP degradabilities of MBM calculated at outflow rate 2 and 5% were consistent with findings of Kamalak *et al.* (2005) but lower than that observed by Souza *et al.* (2000). This difference possibly due to differences in pore size and particle size, raw materials and manufacturing process such as the length of time that raw meat and bone are stored before processing, whether or not type of dryer used, duration of heating, etc. (Harris and Staples, 1992).

γ-irradiation at 15 kGy had only a slight effect on the washout and potentially degradable fractions of DM and CP, which is consistent with Lee *et al.* (2005) which showed that γ-irradiation below 16 kGy was not effective in formation of high molecular weight aggregates but increased them at higher doses. The γ-irradiation decreased the effective CP degradability of MBM is in agreement with Shawrang *et al.* (2008), who showed that γ-irradiation of canola meal at doses of 25, 50 and 75 kGy decreased the effective CP degradability of canola meal at a ruminal outflow rate of 0.05 h^{-1} by 19, 27 and 32%, respectively. Decreasing CP degradability as a result of irradiation is due to the occurrence of cross-linking of polypeptide chains, denaturation, protein aggregation and oxidation of protein by oxygen radicals (Zabielski *et al.*, 1984; Davies and Delsignore, 1987; Nisizawa, 1988; Gaber, 2005; Abu *et al.*, 2006). This agrees with Gaber (2005), who found that hydroxyl and superoxide anion radicals that are generated by radiation could modify the molecular properties of proteins by cross-linking and aggregation of polypeptide chains. Cross-linking of proteins was also observed by Ebrahimi *et al.* (2009) and Shawrang *et al.* (2008) by γ-irradiating of canola meal and canola seed. Moreover, Ressouany *et al.* (1998) demonstrated that γ-irradiation of soybean protein at 32 kGy increased the protein molecular weight from 60-2000 kDa. Cross-linking results in formation of chemical bonds between two adjacent protein molecules. Cross-linked proteins are hydrophobic making them more compact and could pass from rumen to the intestine undegraded. DM and CP disappearance from nylon bags incubated in the rumen increased with increasing incubation time. Irradiation decreased DM and CP disappearance from nylon bags within each time of rumen incubation. Irradiation induces unfolding of protein structures and their denaturation, thereby increasing surface hydrophobicity of proteins by exposing non-polar groups (Woods and Pichaev, 1994).

Table 2: Rumen degradation parameters of dry matter and crude protein and *in vitro* crude protein digestibility of undegraded protein of untreated and γ-irradiated Meat and Bone Meal (MBM)

		γ-irradiated MBM (kGy)			
Parameters	Untreated MBM	15	30	45	SEM
Dry matter					
a (g kg^{-1})	234.0	234.0	213.0	175.0	5.5.00
b (g kg^{-1})	421.0	428.0	456.0	482.0	13.5.0
a + b (g kg^{-1})	665.0	662.0	669.0	657.0	15.6.0
c h^{-1}	0.041	0.039	0.035	0.034	0.0028
Effective rumen degradation (g kg^{-1})					
0.02 h^{-1}	516.0	515.0	501.0	478.0	10.800
0.05 h^{-1}	424.0	420.0	399.0	370.0	9.7.00
0.08 h^{-1}	377.0	373.0	350.0	318.0	8.6000
Crude protein					
a (g kg^{-1})	252.0	261.0	226.0	191.0	8.3000
b (g kg^{-1})	438.0	430.0	471.0	513.0	15.800
a + b (g kg^{-1})	690.0	691.0	697.0	704.0	16.300
c h^{-1}	0.049	0.041	0.038	0.034	0.0028
Effective rumen degradation (g kg^{-1})					
0.02 h^{-1}	563.0	547.0	531.0	511.0	12.200
0.05 h^{-1}	469.0	453.0	426.0	396.0	11.700
0.08 h^{-1}	419.0	405.0	375.0	342.0	11.100
***In vitro* crude protein**					
Digistibility (g kg^{-1})	601.0	603.0	688.0	710.0	7.000

a, the washout fraction; b, the potentially degradable fraction; c, the rate of degradation

When secondary and tertiary structures of a protein are unfolded (Gaber, 2005), proteins may be converted to high molecular weight aggregates due to generation of interprotein cross-linkages, hydrophobic and electrostatic interactions and formation of disulfide bonds (Garrison, 1987; Davies and Delsignore, 1987; Lee Maire *et al*., 1990). Any amino acid radical formed within a peptide chain could cross-link with an amino acid radical in another protein. Hydrophobic groups interact and reduce water binding. Moreover, hydrophobic interactions lead to aggregation followed by coagulation and precipitation (Englard and Seifter, 1990), probably reducing ruminal CP degradability.

Effects of γ-irradiation on *in vitro* CP digestibility of MBM: γ-irradiation increased ($p<0.05$) *in vitro* CP digestibility of MBM (Table 2). γ-irradiation at doses of 30 and 45 kGy, increased *in vitro* CP digestibility of undegraded CP remaining in bags incubated for 16 h in the rumen by 13 and 15%, respectively. The CP digestibility value of untreated MBM was 601 g kg^{-1}. Low CP digestibility of MBM has been associated with the content of ossein and collagen, proteins typically found in bone and connective tissue, respectively (Howie *et al*., 1996). The CP digestibility value was similar to that reported by Maiga *et al.* (1996) and lower than that reported by England *et al.* (1997). The value of 560 g kg^{-1} was reported by Howie *et al.* (1996). Discrepancies in reported CP digestibility values may be due to differences in methods of estimating CP digestibility, raw materials and manufacturing process. Protein denaturation as a result of γ-irradiation increased *in vitro* CP digestibility of irradiated MBM.

γ-irradiation at doses of 30 and 45 kGy, increased CP digestibility of MBM by 13 and 15%, respectively. The results are consistent with Shawrang *et al.* (2007, 2008), who reported that γ-irradiation increased intestinal CP digestibility of soybean and canola meals. Shawrang *et al.* (2008) reported that γ-irradiation at doses of 25, 50 and 75 kGy increased intestinal CP digestibility of canola meal by 4, 13 and 20%, respectively.

Moreover, Ebrahimi *et al.* (2009) showed that γ-irradiation at doses of 30 and 45 kGy increased *in vitro* CP digestibility of canola seed by 15 and 21%, respectively. γ-irradiation may induce unfolding of the protein and its denaturation, thereby exposing hydrophobic amino acids (especially aromatics) that are position groups for active sites of pepsin and trypsin enzymes (Murray *et al*., 2003; Abu *et al*., 2006). Moreover, with modification in the secondary and tertiary structures of protein by γ-irradiation, more peptide bonds are exposed to proteolytic enzymes (Fombang *et al*., 2005).

CONCLUSION

Results suggest that γ-irradiation of MBM decreased its ruminal CP degradability by cross-linking of the polypeptide chains. Moreover, γ-irradiation increased *in vitro* CP digestibility of MBM, but further investigation is needed to clarify effects of other doses of γ-irradiation on CP digestibility and the benefits of this processing to animals.

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