South African Journal of Animal Science 2023, 53 (No. 1)

In vitro ruminal fermentation parameters of canola meal protein in response to incremental doses of gamma irradiation

M Sekali,[12#] V Mlambo,[3] U Marume,[12] M Mathuthu[4]

[1]Department of Animal Sciences, Faculty of Natural and Agricultural Science, North-West University, P/Bag x2046, Mmabatho, 2735, South Africa

[2]Food Security and Safety Niche area, Faculty of Natural and Agricultural Science, North-West University, P/Bag x2046, Mmabatho, 2735, South Africa

[3]School of Agricultural Sciences, Faculty of Agriculture and Natural Sciences, University of Mpumalanga, P/Bag x11283, Mbombela, 1200, South Africa

[4]Centre for Applied Radiation Science and Technology, Faculty of Natural and Agricultural Science, North-West University, P Bag x2046, Mmabatho, 2735, South Africa

(Submitted 8 September 2020; Accepted 13 June 2021; Published 11 April 2023)

Abstract

Rapid ruminal degradation of canola meal (CM) limits its feed value for high-producing ruminants. Attempts to reduce ruminal degradability of CM through gamma irradiation have generated conflicting results. While this strategy has the potential to reduce CM degradability, the optimal radiation dose is unknown for this valuable co-product. Therefore, this *in vitro* ruminal fermentation study was designed to evaluate the efficacy of gamma irradiation to protect CM protein from ruminal degradation. Canola meal was irradiated at 0 (CM0), 15 (CM15), 30 (CM30), 45 (CM45), 60 (CM65), 75 (CM75), and 90 kGy (CM90). Irradiated CM was then analysed for proximate composition and incubated with rumen fluid to determine *in vitro* degradability of dry matter (DMD) and nitrogen (ND). The data were evaluated for linear and quadratic effects using response surface regression analysis. Neutral detergent fibre and acid detergent fibre linearly decreased as irradiation dosage increased. Quadratic responses were observed for total nitrogen (N) content, DMD12, and DMD36 in response to increasing irradiation dosage. Gamma irradiation linearly increased the rapidly soluble fraction (*a*) and effective degradability (ED) of dry matter. There were no irradiation effects on ND12, ND36, ND48, fractional rate constant (*c*), and potential degradability, but significant quadratic trends were observed for ND24, *a*, slowly degradable fraction (*b*), and ED of N. It was concluded that although gamma irradiation altered the chemical composition of CM, it was not an effective method to protect CM from extensive ruminal degradation.

Keywords: canola meal, dry matter degradability, protein degradability
[#]Corresponding author: msekali29@gmail.com

Introduction

The high demand for vegetable oils for human consumption generates large quantities of protein-rich oil extract residues (Sá et al., 2021). Soybean (*Glycine max)* and canola (*Brassica napus* and *Brassica campestris/rapa*) seeds are some of the most common sources of edible oils. Soybeans tend to be more expensive because they are directly used as food for humans and feed for animals. Agronomical requirements for soybean production in South Africa also tend to be very high resulting in high costs of production. On the other hand, canola meal has no direct food value for humans and thus tends to be less expensive. When used in ruminant diets, canola meal is highly palatable (Heendeniya *et al.*, 2012) and has a well-balanced amino acid profile (Hickling, 2008). Unfortunately, canola meal is not an effective source of amino acids post-ruminally because its protein is highly degradable in the rumen (44.3–74%) (Wright *et al.*, 2005). Indeed, canola meal has been shown to contain highly soluble proteins (Newkirk, 2009), which reduces its feeding value, especially for high-producing ruminants. Therefore, it is imperative that strategies to reduce rumen degradability of canola protein be identified and evaluated in order to improve its efficiency of utilization as an alternative protein source to

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 2221-4062 (online)
Publisher: South African Society for Animal Science
http://dx.doi.org/10.4314/sajas.v53i1.12

soybean meal. To this end, various chemical and physical methods have been used to reduce the rate of degradation of protein sources in the rumen (Tuncer & Sacackli, 2003; Yoruk *et al.*, 2006; Eghbali *et al.*, 2011). The physical methods include heat treatment (Eghbali *et al.*, 2011) and irradiation (Golshan *et al*., 2019). The rupturing of covalent bonds and formation of protein free radicals, oxidation of amino acids by oxygen radicals that are formed in the radiolysis water, and formation of disulphide bonds are thought to be some of the mechanisms through which irradiation reduces the rumen degradable protein fraction (Lee *et al.*, 2005; Afify *et al.*, 2011). While some studies have reported no changes in chemical composition of irradiated protein sources (Shawrang *et al.*, 2007; Ebrahimi *et al.*, 2009; Ebrahimi-Mahmoudabad & Taghinejad-Roudbaneh, 2010; Anwar, 2015), others have reported higher DM (Akbarian *et al.*, 2014), lower NDF content, and reduced nitrogen degradation by rumen microbes (Ghanbari *et al.*, 2012). These changes are attributed to radiolysis of the water content of the protein source (Hamza *et al.*, 2012), reduced protein solubility, disruption of hemicellulose and cell wall-associated true protein (Akbarian *et al.*, 2014), and a decrease in non-starch polysaccharides caused by cleavage of glycosidic bonds (Moradi *et al.*, 2015). The discordance in reported effects of irradiation on chemical and biophysical properties of proteins is mostly due to variation in applied doses. Consequently, it is imperative that correct doses be applied to prevent overprotection of proteins from rumen microbial fermentation, which may lead to reduced digestibility of the protein in the small intestines. Therefore, the objective of this study was to determine the optimal dose of gamma radiation for canola meal based on changes in chemical properties and *in vitro* rumen protein degradability.

Material and methods

All animal handling practices were according to the recommendations of the National Society for the Prevention of Cruelty to Animals (NSPCA) in South Africa. The experimental procedures were approved by the University Ethics Committee, North-West University, South Africa (Ethical Clearance: NWU-00523-16-A9). Gamma irradiation of canola meal (CM) was done at Hepro (Cape) Pty Ltd, Cape Town, South Africa. Chemical analyses and *in vitro* ruminal fermentation experiments were conducted at North-West University Farm Laboratories, Mafikeng, South Africa. Soybean meal was purchased from Opti-feeds (Pvt, Ltd) (Lichtenburg) and canola meal was purchased from Southern Oil (Pty) Ltd Company in Western Cape, South Africa. Both meals were ground to pass through a 2-mm sieve using a Wiley Mill (Arthur H. Thomas Company, Model ED5, Philadelphia, PA).

Twenty-four milled CM samples (50 g each) were separately weighed into brown paper bags that were subsequently sealed. The bags were then independently irradiated with gamma rays (four bags per radiation dose) generated by a Cobalt 60 radiator (Gamma Cell 220, Ottawa, Canada) with a dose rate of 33 Gy/min at 25 °C and 77.7% humidity. The cobalt 60 was packed inside double-encapsulated, stainless-steel rods. The samples were placed into pallets measuring 1 m × 1.2 m × 1.5 m. A batch number was assigned, which also indicated the rate of dose. A gamma sensitive label (DETEX), which changed colour (yellow to red) was attached to each of the bag labels and used as a qualitative measure for gamma ray exposure. A dosimeter was then placed on the pallets and was read in a calibrated spectrophotometer to provide a quantitative measure of the dose absorbed by the meal. Canola meal was evenly irradiated at doses of 15, 30, 45, 60, 75, and 90 kGy with four replicate samples being independently exposed to each dose of gamma radiation. Irradiated samples were then left for 2 hours to air-equilibrate. Four control samples of CM were left at room temperature and were denoted to have been irradiated at 0 kGy. Untreated and irradiated canola meal samples were subjected to chemical analyses to quantify dry matter (DM, AOAC, 2005; method no. 930.15), ash (AOAC, 2005; method no. 942.05), and total nitrogen content (AOAC, 2005; method no. 976.05). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to Van Soest *et al.* (1991) using an ANKOM 220 Fibre Analyzer (ANKOM Technology Corporation, NY, USA). Heat-stable bacterial α-amylase, but not sodium sulphite (Na_2SO_3), was used for NDF analysis. The fibre fractions were then expressed in g/kg DM inclusive of residual ash.

In vitro ruminal degradability of CM was determined using the filter bag technique. ANKOM F57 filter bags (57 µm pore size) (ANKOM, Macedon, NY) were rinsed in acetone and allowed to air-dry. Approximately 0.45–5.0 g of CM samples were weighed into the bags, which were subsequently heat-sealed and placed into the digestion jars of the ANKOM^{II} Daisy incubator (ANKOM Technology). A buffer was prepared according to ANKOM Technology Method No. 3 and added (1600 ml) to each of the four digestion jars, which were incubated (39.5 ± 0.5 °C) and continuously rotated in the incubator. Rumen fluid inoculum was collected in the morning before feeding from a cannulated Bonsmara cow. The animal had been on a diet of blue buffalo grass supplemented with a commercial beef concentrate. The inoculum was collected from the rumen directly into a pre-warmed thermos flask. Rumen inoculum was then emptied into a blender and homogenized (15 000 rpm) for 30 seconds to dislodge microbes attached to the mat. The fluid was then filtered through three layers of cheesecloth. Rumen fluid was prepared under constant purging with carbon dioxide gas to maintain anaerobic

conditions. Homogenized and filtered rumen inoculum (350 ml) was then added into each jar. Digestion jars were also continuously purged with a stream of CO_2 gas to maintain anaerobic conditions. ANKOM bags were then incubated for 0, 2, 4, 8, 12, 24, 36, 48, and 72 hours. Three runs of ANKOM incubations were carried out for every independent replicate. At the end of each incubation period, the bags were withdrawn and rinsed with cold distilled water and refluxed with neutral detergent solution for 1 hour using the ANKOM^{2000} Fibre Analyzer. The bags were then dried at 105 °C in an oven until constant weight, which was then recorded. The zero-hour bags were not incubated with the rumen fluid but were washed with cold water and refluxed in neutral detergent solution as described above. Nitrogen content of dried samples was determined as already described for total nitrogen. Nitrogen degradability was estimated as the loss of total N upon incubation. Dry matter and nitrogen degradability parameters were estimated by fitting the degradation data to the following equation using SAS software (2010):

$$Y = a + b\,(1 - e^{-ct})$$

where *Y* = degraded fraction after *t* hours of incubation; *a* = soluble or rapidly degradable fraction; *b* = insoluble or slowly degradable fraction; *c* = fractional rate constant of degradation of *b* (%/h); *t* = incubation time (0, 2, 4, 8, 12, 24, 36, 48, and 72 hours), and e = base for natural logarithm. Potential degradability (PD) was calculated as the sum of fractions *a* and *b*. Effective rumen degradability value was calculated as:

$$ED = a + \frac{b \times c}{k + c}$$

where *a*, *b*, and c are the Orskov–McDonald parameters defined above, while *k* is the outflow rate, assumed to be 5 %/h.

Chemical composition and *in vitro* ruminal fermentation data were evaluated for linear and quadratic effects using polynomial contrasts. Response surface regression analysis (Proc RSREG; SAS, 2010) was applied to estimate the optimum irradiation dose for CM, according to the following quadratic model:

$$y = c\ + bx + ax^2,$$

where *y* = response variable; *a* and *b* are the coefficients of the quadratic equation; *c* is the intercept; *x* is gamma irradiation dose (kGy), and *-b/2a* is the *x* value for maximum or minimum response. The effect of irradiation level on DM and N degradability parameters was evaluated by analysis of variance using the model:

$$Y = \mu\ +\ a_i\ +\ e_{ij},$$

where μ = the overall mean; α_i = the effect of gamma irradiation; *and* e_{ij}: the residual error. Where gamma irradiation was detected to have a significant effect, means were separated using Tukey's procedure. Statistical significance was declared at $P < 0.05$.

Results

The chemical composition of CM irradiated with different doses of gamma rays is presented in Table 1. As gamma irradiation doses increased, quadratic responses were observed for laboratory DM and OM content of canola meal. Neutral detergent fibre ($y = 287.92\ (\pm 4.46) - 0.93\ (\pm 0.24)x$; P =0.0001; R^2 =0.77) and ADF ($y = 237.34\ (\pm 3.87) - 0.25\ (\pm 0.21)x$; P =0.03; R^2 =0.23) content of CM decreased linearly in response to increasing irradiation doses. Acid detergent fibre content was reduced from 239.41 g/kg in untreated CM to 223.20 g/kg in CM irradiated with the highest dose (CM90). The N content exhibited a quadratic ($y = 383.06\ (\pm\ 7.91) -\ 2.10\ (\pm\ 0.43)x\ +\ 0.02\ (\pm 0.004)x^2$; P =0.001; R^2 =0.60) response to increasing irradiation doses. The highest nitrogen content (5.49 g/kg) was observed in CM irradiated at 60 kGy (CM60). There were neither linear nor quadratic effects of irradiation doses on the ash content of canola meal.

Table 1 Chemical composition (g/kg DM, unless otherwise stated) of untreated and γ-irradiated canola meal (CM)

	Untreated CM	γ-irradiated CM						SEM	Contrasts	
		15 kGy	30 kGy	45 kGy	60 kGy	75 kGy	90 kGy		Linear	Quadratic
DM (g/kg)	939.9	926.9	926.1	928.6	929.2	929.6	936.5	1.99	NS	**
Ash	76.1	77.8	76.8	76.1	75.7	77.1	76.9	0.42	NS	NS
OM	863.8	849.1	849.2	852.5	853.5	852.5	859.6	2.11	NS	**
NDF	292.5	265.8	254.0	253.8	246.3	250.5	234.2	4.85	**	NS
ADF	239.4	231.5	227.4	229.7	227.8	230.5	223.2	3.93	*	NS
Nitrogen	63.8	52.1	52.8	53.3	54.9	54.4	54.3	0.35	**	**

NS: not significant; * *P* <0.05; ** *P* <0.01; SEM: standard error of the mean
DM = dry matter; OM = organic matter; NDF = neutral detergent fibre; ADF = acid detergent fibre

Gamma irradiation quadratically influenced dry matter degradability at 12 hours ($y = 511.64\ (\pm 6.16) - 0.84\ (\pm 0.32)x + 0.01\ (\pm 0.003)x^2$; *P* =0.01; R^2 =0.53) and 36 hours ($y = 798.53(\pm 6.38) - 0.64\ (\pm 0.33)x + 0.01\ (\pm 0.003)x^2$; *P* =0.03; R^2 =0.28) post-inoculation with rumen fluid (Table 2). However, neither linear nor quadratic trends were observed for dry matter degradability at 24- and 48-hours post-inoculation.

Table 2 *In vitro* ruminal dry matter degradability (g/kg) of untreated and γ-irradiated canola meal (CM) at various incubation times

Time (h)	Untreated CM	γ-irradiated CM						SEM	Contrasts	
		15 kGy	30 kGy	45 kGy	60 kGy	75 kGy	90 kGy		Linear	Quadratic
12	514.20	495.01	499.13	504.56	502.48	511.92	536.13	7.83	*	**
24	755.80	731.41	756.58	767.86	755.05	733.27	739.43	11.11	NS	NS
36	802.63	782.31	789.99	790.61	784.41	803.71	805.92	7.88	NS	*
48	812.45	807.64	802.91	653.44	812.79	807.60	816.55	63.29	NS	NS

NS: not significant; * *P* <0.05; ** *P* <0.01; SEM: standard error of the mean

Table 3 shows the *in vitro* ruminal dry matter degradability parameters of canola meal in response to incremental doses of γ irradiation. There was a linear increase in the *a* ($y = 253.94\ (\pm 10.02) + 0.89\ (\pm 0.52)x$; *P* =0.001; R^2 =0.50) and ED ($y = 568.81\ (\pm 5.36) + 0.04\ (0.28)x$; *P* =0.003; R^2 =0.40) fractions of dry matter in response to incremental levels of irradiation doses. Gamma irradiation increased the *a* fraction from 253.30 g/kg in the control to 304.40 g/kg in the substrate receiving the highest irradiation (CM90). There were neither linear nor quadratic trends for the slowly degradable fraction (*b*), the rate of degradation (*c*), and the potential degradability of dry matter as gamma irradiation doses increased.

Table 3 *In vitro* ruminal dry matter degradation parameters (g/kg DM, unless otherwise stated) of untreated and γ-irradiated canola meal (CM)

	Untreated CM	γ-irradiated CM						SEM	Contrasts	
		15 kGy	30 kGy	45 kGy	60 kGy	75 kGy	90 kGy		Linear	Quadratic
a	252.3	269.9	280.5	280.3	293.4	312.8	304.4	13.42	*	NS
b	604.0	583.6	568.1	512.2	558.0	553.1	368.1	79.06	NS	NS
c (*/h*)	0.05	0.05	0.06	0.07	0.05	0.05	0.05	0.01	NS	NS
PD	856.4	853.5	848.6	792.5	851.4	866.0	672.5	80.36	NS	NS
ED	568.3	568.9	578.4	569.0	583.4	584.1	493.2	40.87	*	NS

NS: not significant; * *P* <0.05; SEM: standard error of the mean
a = washing loss or rapidly fermentable fraction; *b* = degradable part of the insoluble fraction; *c* = degradation rate of fraction *b*; *PD* = potential degradability calculated as the sum of parameters *a* and *b*; *ED* = effective digestibility, calculated as: $a + \frac{b \times c}{k+c}$, and *k* is the outflow rate, which was assumed to be 5%/hour

Nitrogen degradability after 24 hours of incubation quadratically ($y = 785.89\ (\pm 20.18) + 3.60\ (\pm 1.50)x - 0.04\ (\pm 0.04)x^2$; *P* =0.002; R^2 =0.41) responded to incremental levels of irradiation doses; however, there

were neither linear nor quadratic responses for nitrogen degradability values at 12-, 36-, and 48-hours post-inoculation (Table 4).

Table 4 *In vitro* ruminal nitrogen degradability (g/kg N) of untreated and γ-irradiated canola meal (CM) at various incubation times

Time (h)	Untreated CM	γ-irradiated CM						SEM	Contrasts	
		15 kGy	30 kGy	45 kGy	60 kGy	75 kGy	90 kGy		Linear	Quadratic
12	513.37	446.35	540.01	461.03	463.47	464.75	473.12	42.82	NS	NS
24	782.44	827.09	864.28	884.65	865.46	789.34	813.91	23.16	NS	**
36	896.77	912.86	924.21	924.27	913.00	912.99	919.12	12.86	NS	NS
48	934.75	933.17	878.11	891.32	943.56	939.90	937.05	38.30	NS	NS

NS: not significant; ** *P* <0.01; SEM: standard error of the mean

While quadratic responses were observed for *a* ($y = 134.45\ (\pm 16.59) + 2.59\ (\pm 0.86)x - 0.03\ (\pm 0.01)x^2$; *P* =0.01; R^2 =0.33), *b* ($y = 858.39\ (\pm 15.33) - 2.88\ (\pm 0.80)x + 0.03\ (\pm 0.008)x^2$; *P* =0.001; R^2 =0.46), and ED ($y = 577.19\ (\pm 8.62) + 1.60\ (\pm 0.45)x - 0.02\ (\pm 0.005)x^2$; *P* =0.002; R^2 =0.43) fractions of nitrogen in response to graded levels of gamma irradiation, no significant relationships were observed for *c* and PD fractions (Table 5).

Table 5 *In vitro* ruminal N degradation parameters (g/kg N, unless otherwise stated) of untreated and γ-irradiated canola meal (CM)

	Untreated CM	γ-irradiated CM						SEM	Contrasts	
		15 kGy	30 kGy	45 kGy	60 kGy	75 kGy	90 kGy		Linear	Quadratic
a	124.3	193.6	172.8	189.6	201.1	182.2	151.6	18.30	NS	**
b	864.9	808.3	809.4	794.6	803.1	831.7	862.8	18.06	NS	**
c (*/h*)	0.05	0.05	0.06	0.06	0.05	0.05	0.05	0.01	NS	NS
PD	989.3	1001.9	982.2	984.2	1004.2	1014.0	1014.3	15.89	NS	NS
ED	575.0	599.9	609.9	612.0	613.2	588.6	581.5	10.11	NS	**

NS: not significant; ** *P* <0.01; SEM: standard error of the mean
a = washing loss or rapidly fermentable fraction; *b* = degradable part of the insoluble fraction; *c* = degradation rate of fraction *b*; *PD* = potential degradability calculated as the sum of parameters *a* and *b*; *ED* = effective digestibility, calculated as: $a + \frac{b \times c}{k+c}$, and *k* is the outflow rate, which was assumed to be 5%/hour

Discussion

In some studies, electron and gamma irradiation did not affect the chemical composition of soybeans and canola seeds (Shawrang *et al*., 2007; Taghinejad *et al.*, 2009a; Ebrahimi *et al.*, 2009; Ebrahimi-Mahmoudabad & Taghinejad-Roudbaneh, 2010; Anwar *et al*., 2015). However, in the current study, and in agreement with Golsham *et al.* (2019), gamma irradiation increased DM content, which can be attributed to radiolysis of moisture in CM (Hamza *et al.*, 2012). Increased dry matter content as a result of irradiation has also been reported by Kaya *et al.* (2016) for oak nut. It is important to note that studies that have reported no differences in the chemical composition of soybean (Ebrahimi-Mahmoudabad & Taghinejad-Roudbaneh, 2010) and canola seeds (Anwar *et al*., 2015) employed a maximum dose of 75 kGy as opposed to the 90 kGy dose used in this study.

Reduction in NDF and ADF content of CM upon gamma irradiation agrees with previous findings in a variety of agricultural by-products (Akbarian *et al.*, 2014; Nayefi *et al.*, 2014; Moradi *et al.*, 2015) when exposed to both gamma and electron beam irradiation. According to Akbarian *et al.* (2014), irradiation disrupts hemicellulose and releases cell wall-bound protein, which would otherwise be insoluble in neutral detergent solution used in the determination of NDF. Changes in fibre due to irradiation have also been associated with a decrease in non-starch polysaccharide content caused by the cleavage of glycosidic bonds, leading to the formation of sugars such as glucose and maltose (Moradi *et al.*, 2015). The observed increase in N concentration in gamma-irradiated CM could simply be due to the reduction in moisture content. This is because N content is expressed on a DM basis; a reduction in moisture content therefore results in an increase in N concentration despite there being no change in the absolute amount of N in radiated samples.

Irradiation produces radicals due to radiolysis of water molecules (El-Beltagi *et al*., 2011). The reaction of these radicals with protein molecules causes damage (Filali-Mouhim *et al.*, 2000; Afify *et al.*, 2011) leading to breakdown of β-bonds, thereby opening an hydroglucose ring or breaking the glycoside bond and carboxyl groups produced in the process (Shahbazi *et al.*, 2008). Gamma irradiation increased the soluble DM fraction (*a*) of canola meal, in contrast to reports by Sadeghi & Shawrang (2006), Sadeghi & Shawrang (2007), and Ghanbari *et al.* (2015). These scholars reported a decrease in the *a* fraction and an increase in the *b* fraction of canola meal, cotton seed meal, and sunflower meal dry matter. However, Moradi *et al.* (2015) reported an increase in the *a* and *b* fractions of pistachio by-product dry matter. An increase in the *a* fraction reported in the current study could be ascribed to reduced NDF content of irradiated CM (Moradi *et al.*, 2015), which may have resulted in faster rumen degradability of the substrate. Electron beam irradiation at doses of 25, 50, and 75 kGy had no effect on the rate of degradation (*c*) of the slowly degradable fraction of sunflower meal (Ghanbari *et al.*, 2015), a finding corroborated in the current study. Irradiation of canola meal did not affect the potential degradability (*a* + *b*) of dry matter in agreement with reports by Taghinejad-Roudbaneh *et al.* (2010) and Moradi *et al.* (2015). A positive, linear increase in ED in response to irradiation doses could be due to an increase in the *a* fraction of irradiated canola meal. Effective degradability of dry matter in this study was higher than that reported for cotton seed (Ebrahimi-Mahmoudabad & Taghinejad-Roudbaneh, 2011; Taghinejad-Roudbaneh *et al.*, 2016) but lower than those reported for soybean and canola seed (Taghinejad *et al.*, 2009b; Ebrahimi-Mahmoudabad & Taghinejad-Roudbaneh, 2010). In their study, Taghinejad-Roudbaneh *et al.* (2010) reported that effective degradability of dry matter for irradiated canola meal decreased with increasing irradiation dose, findings that were contradicted by the current study.

Contrary to expectations, gamma irradiation did not affect ruminal nitrogen degradability of canola meal at 12, 36, and 48h post-inoculation. Irradiation is reported to reduce N degradability by producing oxygen radicals that cleave disulphide bonds and crosslinks that are involved in secondary and tertiary protein structures (Taghinejad-Roudbaneh *et al.*, 2010). This disruption unfolds the protein structure, exposing non-polar groups, thus increasing the surface hydrophobicity of protein, leading to high molecular weight aggregates (Lee *et al.*, 2005). This favours protein aggregation, coagulation, and precipitation, thereby reducing its degradability (Ebrahimi *et al.*, 2009). Findings in the current study suggest that irradiation doses may not have been high enough to reduce CM protein degradability. The current findings are similar to those reported by Ghanbari *et al.* (2015) in sunflower and cottonseed meals irradiated at 25, 50, and 75 kGy. In contrast, irradiation of canola meal (30–45 kGy) decreased the *a* fraction while increasing the *b* fraction (Taghinejad *et al.*, 2009a; 2010). Differences in the quaternary structure of protein substrates have been observed following irradiation. For some proteins, irradiation has been reported to induce unfolding in proteins due to deamination, thereby increasing the solubility of a protein molecule (Dogbevi *et al.,* 2000). For low-producing ruminants, increased solubility of nitrogen is desirable because it enhances nitrogen availability for ruminal microbial protein synthesis.

Conclusion

Gamma irradiation of canola meal at doses up to 90 KGy did not reduce the rate of ruminal degradation of canola protein and thus cannot be used as a strategy to increase by-pass protein in ruminants. It is therefore, recommended that other physical treatment methods or higher irradiation doses be investigated.

Acknowledgements

We wish to thank North-West University for providing laboratory equipment and consumables as well as technical support.

Authors' contribution

MS, MM, and VM planned the experiment; VM, MM, and UM supervised the experiment. MS conducted the experiment, collected the experimental data. MS and VM analysed data, wrote the first draft of the manuscript. UM, VM, MM assisted in statistical analysis and interpretation of the results. MS interpreted the results. All authors critically reviewed the manuscript for intellectual content and gave final approval for the version to be published.

Conflicts of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

References

Afify, A.E.M.R., Rashed, M.M., Mahmoud, E.A., & El-Beltagi, H.S., 2011. Effect of gamma radiation on protein profile, protein fraction, and solubility of three oil seeds: soybean, peanut and sesame. Not. Bot. Horti. Agrob. 39, 90-98.

Akbarian, A., Ghasemi, E., Khorvash, M., Ghorbani, G.R., Dehghan-Banadaky, M., Shawrang, P., & Ghaffari, H.M., 2014. Effects of roasting and electron beam irradiating on protein characteristics, ruminal degradability, and intestinal digestibility of soybean and the performance of dairy cows. Livest. Sci. 168, 45-52.

Anwar, M.M, Ali, S.E., & Nasr, E.H., 2015. Improving the nutritional value of canola seed by gamma irradiation. J. Radiat. Res. Appl. Sci. 8, 328-333.

AOAC (Association of Analytical Chemists), 2005. Official Methods of Analysis. 18th ed. Gaithersburg, USA.

Dogbevi, M.K., Vachon, C., & Lacroix, M., 2000. Effect of gamma irradiation on the microbiological quality and on the functional properties of proteins in dry red kidney beans (*Phaseolus vulgaris*). Rad. Phys. Chem. 57, 265-268.

Ebrahimi, S.R., Nikkhah, A., Sadeghi, A.A., & Raisali, G., 2009. Chemical composition, secondary compounds, ruminal degradation, and *in vitro* crude protein digestibility of gamma irradiated canola seed. Anim. Feed Sci. Technol. 151, 184-193.

Ebrahimi-Mahmoudabad, S.R, & Taghinejad-Roudbaneh, M., 2011. Investigation of electron beam irradiation effects on anti-nutritional factors, chemical composition, and digestion kinetics of whole cottonseed, soybean, and canola seeds. Radiat. Phys. Chem. 80, 1441-1447.

Eghbali, M., Kafilzadeh, F., Hozhabri, F., Afshar, S., & Kazemi-Bonchenari, M., 2011. Treating canola meal changes in *in situ* degradation, nutrient apparent digestibility, and protein fractions in sheep. Small Rumin. Res. 96, 136-139.

El-Beltagi, H.S., Ahmed, O.K., & El-Desouky, W., 2011. Effect of low doses γ-irradiation on oxidative stress and secondary metabolites production of Rosemary (*Rosmarinus officinalis* L.) callus culture. Radiat. Phys. Chem. 80, 968-976.

Filali-Mouhim, A., Audette, M., St-Louis, M., Thauvette, L., Denoroy, L., Penin, F., Cho, Y., & Song, K.B., 2000. Effect of g-irradiation on the molecular properties of BSA and β-lactoglobolin. J. Biochem. Mol. Biol. 33, 133-137.

Ghanbari, F., Ghoorchi, T., Shawrang, P., Mansouri, H., & Torbati-Nejad, N.M., 2015. Improving the nutritional value of sunflower meal by electron beam and gamma ray irradiations. Iran. J. Appl. Anim. Sci. 5, 21-28.

Ghanbari, F., Ghoorchi, T., Shawrang, P., Mansouri, H., & Torbati-Nejad, N.M., 2012. Comparison of electron beam and gamma ray irradiations effects on ruminal crude protein and amino acid degradation kinetics, and *in vitro* digestibility of cottonseed meal. Radiat. Phys. Chem. 81, 672-678.

Golshan, S., Pirmohammadi, R., & Khalilvandi-Behroozyar, H., 2019. Microwave irradiation of whole soybeans in ruminant nutrition: Protein and carbohydrate metabolism *in vitro* and *in situ*. Vet. Res. Forum. 10, 343-350.

Hamza, R.G., Afifi, S., Abdel-Ghaffar, A.R.B., & Borai, I.H., 2012. Effect of gamma irradiation or/and extrusion on the nutritional value of soy flour. Biochem. Anal. Biochem. 1, 118. doi.org/10.4172/2161-1009.1000118.

Heendeniya, R.G., Christensen, D.A., Maenz, D.D., McKinnon, J.J., & Yu, P., 2012. Protein fractionation by-product from canola meal for dairy cattle. J. Dairy Sci. 95, 4488-4500.

Hickling, D., 2008. Maximized utilization of canola co-products in livestock industry. In 29th Proceedings of the Western Nutrition Conference. University of Alberta: Edmonton. p. 3-14.

Kaya, E., Atalay, A.I., Kurt, O., Zangana, D.N., Salih, M.J.M., & Kamalak, A., 2016. Determination of effects of microwave irradiation on fermentation of oak nut (*Quercus coccifera*) using Hohenheim gas production technique. J. Nat. Sci. 19, 268-271.

Lee, S.L., Lee, M.S., & Song, K.B., 2005. Effect of gamma irradiation on the physicochemical properties of gluten films. Food Chem. 92, 621-625.

Moradi, M., Behgar, M., Afzalzadeh, A., & Norouzian, M.A., 2015. Effects of electron irradiation, sodium hydroxide and polyethylene glycol on the utilization of pistachio by-products by Zandi male lambs. Small Rumin. Res. 127, 1-7.

Nayefi, M., Salari, S., Sari, M., & Behgar, M., 2014. Treatment by gamma or electron radiation decreases cell wall and gossypol content of cottonseed meal. Radiat. Phys. Chem. 99, 23-25.

Newkirk, R., 2009. Canola meal: Feed Industry Guide, 4th ed. Canadian International Grains Institute: Manitoba. p 1-48.

Ørskov, E.R., & McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. J. Agric. Sci. 92, 499-503.

Sá, A.G.A., da Silva, D.C., Pacheco, M.T.B., Moreno, Y.M.F., & Carcio, B.A.M, 2021. Oilseed by-products as plant-based protein sources: Amino acid profile and digestibility. Future Foods 3, 100023.

Sadeghi, A.A., & Shawrang, P., 2006. Effects of microwave irradiation on ruminal degradability and *in vitro* digestibility of canola meal. Anim. Feed Sci. Technol. 127, 45-54.

Sadeghi, A.A., & Shawrang, P., 2007. Effects of microwave irradiation on ruminal protein degradation and intestinal digestibility of cottonseed meal. Livest. Sci. 106, 176-181.

SAS Institute, Inc. 2010. SAS/stat users' guide, release 9.1.2. SAS Institute, Inc., Cary, NC.

Shahbazi, H.R., Sadeghi, A.A., Fazaeli, H., Raisali, G., Chamani, M., & Shawrang, P., 2008. Effect of electron beam irradiation on ruminal NDF and ADF degradation characteristics of barley straw. J. Anim. Vet. Adv. 7, 464-468.

Shawrang, P., Nikkhah, A., Zare-Shahneh, A., Sadeghi, A.A., Raisali, G., & Moradi-Shahrbabak, M.M., 2007. Effects of gamma irradiation on protein degradation of soybean meal in the rumen. Anim. Feed Sci. Technol. 134, 140-151.

Taghinejad, M., Nikkhah, A., Sadeghi, A.A., Raisali, G., & Chamani, M., 2009b. Effects of gamma irradiation on chemical composition, antinutritional factors, ruminal degradation, and *in vitro* protein digestibility of full-fat soybean. Asian-Aust. J. Anim. Sci. 22, 534-541.

Taghinejad, M., Shawrang, P., Rezapour, A., Sadeghi, A.A., & Ebrahimi, S.R., 2009a. Changes in antinutritional factors, ruminal degradability, and *in vitro* protein digestibility of gamma irradiated canola meal. J. Anim. Vet. Adv. 8, 1289-1304.

Taghinejad-Roudbaneh, M., Ebrahimi, S.R., Azizi, S., & Shawrang, P., 2010. Effects of electron beam irradiation on chemical composition, antinutritional factors, ruminal degradation, and *in vitro* protein digestibility of canola meal. Radiat. Phys. Chem. 79, 1264-1269.

Taghinejad-Roudbaneh, M., Kazemi-Bonchenari, M., Salem, A.Z.M., & Kholif, A.E., 2016. Influence of roasting, gamma ray irradiation, and microwaving on ruminal dry matter and crude protein digestion of cottonseed. Ital. J. Anim. Sci. 15, 144-150.

Tuncer, S.D., & Sacakli, P., 2003. Rumen degradability characteristics of xylose treated canola and soybean meals. Anim. Feed Sci. Technol. 107, 211-218.

Van Soest, P.J., Robertson, J.B., & Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597.

Wright, C.F., Von Keyserlingk, M.A.G., Swift, M.L., Fisher, L.J., Shelford. J.A., & Dinn, N.E., 2005. Heat- and lignosulfonate-treated canola meal as a source of ruminal undegradable protein for lactating dairy cows. J. Dairy Sci. 88, 238-243.

Yoruk, M.A., Aksu, T., Gul, M., & Bolat, D., 2006. The effect of soybean meal treated with formaldehyde on amount of protein in the rumen and absorption of amino acid from small intestines. Turk. J. Vet. Anim. Sci. 30, 457- 463.