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Effects of Microwave Irradiation on Ruminal Protein Degradation of Tomato Pomace

N. Maheri-Sis, M. Eghbali-Vaighan, A. Mirza-Aghazadeh, A.A. Shaddel-Telli,
A. Mirzaei-Aghsaghali, A. Aghajanzadeh-Golshani
Department of Animal Science, Shabestar Branch, Islamic Azad University, Shabestar, Iran

Abstract: This study was conducted to evaluate effects of 1000W microwave irradiation for 2.5 and 5 min on ruminal crude protein (CP) degradation parameters of tomato pomace. Nylon bags filled with 5 g of each of untreated or microwave treated tomato pomace, were suspended in the rumen of three fistulated Gezel rams for 0, 4, 8, 12, 16, 24, 36, 48 and 72 h, and obtained data were fitted to a non-linear degradation model to calculate ruminal degradation characteristics. Microwave treatments significantly decreased protein degradability of tomato pomace on different incubation times. Although microwave irradiation decreased ($p<0.05$) the water soluble fraction (*a)* and increased ($p<0.05$) the potentially degradable fraction (*b*) of CP, but constant rate of degradation (*c*), total degradability (*a+b*) and Effective Rumen Degradability of Protein (ERDP) were not significantly affected by microwave irradiation. The effect of 5 min irradiation on decreasing water soluble fraction of CP was higher than that of 2.5 min. In conclusion, microwave irradiation reduced protein solubility in the rumen, but could not significantly affect the extent of escape protein of tomato pomace.

Key words: Microwave irradiation, nylon bags, protein degradation, tomato pomace

INTRODUCTION

Shortage and high price of conventional animal feeds such as Lucerne and grains in arid and semi arid areas of the world like Iran, leads the animal nutrition to effective use of agro-industrial by-products. Developing food industrial factories consequently produced large amount of wastes and by-products which can play an important role in livestock nutrition (Mirzaei-Aghsaghali and Maheri-Sis, 2008; Maheri-Sis *et al*., 2011).

During tomato processing a by-product, known as tomato pomace, is generated. This by-product represents, almost, 4% of the fruit weight (Del Valle *et al*., 2006). Tomato pomace contains 22.6-24.1% protein, 14.5-15.7% fat and 20.8-30.5% fiber on dry matter basis (El-Boushy and Vander-Poel, 1994). Annual production of tomato pomace exceeds 150,000 MT in Iran. The potential use of these wastes in ruminant ration should participate in reducing the shortage of feedstuffs and subsequently increase milk and meat production in Iran (Besharati *et al*., 2008; Aghajanzadeh-Golshani *et al*., 2010). Because of quick spoilage of wet tomato pomace, usually it is drying or ensiling for further using.

There are several effective food and feed processing and drying technologies. Microwave treatment is one of the novel agro-industrial by-product (Zhu *et al*., 2006) and animal feed processing technologies which can affect ruminal degradation and fermentation kinetics of feedstuffs (Sadeghi and Shawrang, 2006a, b; Sadeghi and Shawrang, 2008; Faramarzi Garmroodi *et al*., 2009; Salamatdoust Nobar *et al*., 2010; Maheri Sis *et al*., 2011). Sadeghi and Shawrang (2006a, b) speculated that microwave heating can be decreased ruminal protein degradability of feedstuffs. Zhao *et al*. (2007) reviewed that microwave technology is increasingly playing an important role in drying in the food industry because of its rapid heating rate and ease of use. It also shows considerable potential for preventing mildew in food. In addition, this method of processing does not cause environmental pollution or introduce foreign chemical reagents. Farag *et al*. (1996) illustrated that microwave heating reduced the aflatoxin content considerably in contaminated material. Microwave irradiation has been used recently by Al-Harahsheha *et al*. (2009) for drying tomato pomace as an alternative method. They were found that microwave drying could be used effectively for drying of such waste product by shortening of the drying process time. The drying rate was found to increase with increasing microwave dosage.

Several methods such as *in vivo*, *in situ* and *in vitro* techniques have been used in order to evaluate the nutritive value of feedstuffs (Maheri-Sis *et al*., 2008). The nylon bag (*in situ*) technique, provides a powerful tool for the initial evaluation of feedstuffs and for improving our understanding of the processes of degradation which occur within the rumen. However, it must be remembered that the technique has limitations as well as advantages. There are three important limitations. Firstly, since the sample is confined within the bag it is not exposed to any breakdown due to chewing and rumination. Secondly food

Corresponding Author: N. Maheri-Sis, Department of Animal Science, Shabestar Branch, Islamic Azad University, Shabestar, Iran

would normally be able to leave the rumen once broken down to a suitable particle size. Thirdly, it must be remembered that, strictly speaking, what is actually measured is the breakdown of material to a size small enough to leave the bag and not necessarily a complete degradation to simple chemical compounds. The results must therefore be treated with due caution, and, in general, be used as qualitative indicators of general principles (Ørskov *et al.*, 1980). In spite of these limitations, it is the best method for simulation of real ruminal condition.

The aim of this study was to determine the effects of microwave irradiation on ruminal protein degradation of tomato pomace using nylon bags technique.

MATERIALS AND METHODS

Sample collection and Chemical Analysis: Tomato pomace samples were collected from four tomato processing factories in Shabestar, East Azerbaijan province, Iran. Dry Matter (DM) was determined by drying the samples at 105ºC overnight and ash by igniting the samples in muffle furnace at 525ºC for 8 h. Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1990). Crude Protein (CP) was calculated as Nx6.25. Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were determined by procedures outlined by Goering and Van Soest (1970) with modifications described by Van Soest *et al*. (1991); sulfite was omitted from NDF analysis. Non-fibrous carbohydrate (NFC) was calculated using the equation of NRC (2001); NFC% = 100 – (NDF% + CP% + EE% + Ash %). All experimental procedures (including chemical composition and *in situ* degradation) were conducted at summer 2009 in animal research farm of Islamic Azad University, Shabestar Branch, Shabestar, Iran.

***In situ* degradation procedures:** Three ruminally cannulated Gezel rams (about 55 kg BW) were used to determine in situ degradation characteristics. Rams were housed in individual tie stalls bedded with sawdust. Rams fed diets containing alfalfa hay (70%) and concentrate mixture (30%) at the maintenance levels. Dacron bags (18*9 cm; 40-45 micron pore size) were filled with 5 g dried and ground samples and then incubated in the rumen of rams for the periods of 0, 4, 8, 12, 16, 24, 36, 48, 72 h. After the removal of bags from the rumen, bags were washed in cold water until rinse were clear and dried at 60ºC for 48 h (Karsli and Russell, 2002). Remaining residues were analyzed for DM and CP concentrations. Crude protein were divided into three fractions as follows: 1) The soluble fraction (fraction *a*) determined as CP loss during the washing process, 2). The potentially digestible fraction (fraction *b*) determined as the differences between initial CP content after washing and the amounts of CP recovered after 72 h incubation, 3) The indigestible fraction (fraction *c*) determined as the amount of CP residue recovered after 72 h incubation (Karsli and Russell, 2002).

Rumen degradation kinetics of CP was calculated using the nonlinear model proposed by Ørskov and McDonald (1979):

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

where;

P = Percentage of degradability for response variables at t
t = Time relative to incubation (h)
a = Highly soluble and readily degradable fraction (%)
b = Insoluble and slowly degradable fraction (%)
c = Rate constant for degradation (h^{-1})
e = 2.7182 (Natural logarithm base)

Following determination of these parameters, the effective degradability of CP in tomato pomace was calculated using and equation described by Ørskov and McDonald (1979):

$$ED = a + (b*c)/(c+k)$$

where:

ED = Effective degradability for response variables (%)
a = Highly soluble and readily degradable fraction (%)
b = Insoluble and slowly degradable fraction (%)
c = Rate constant for degradation (h^{-1})
k = Rate constant of passage (h^{-1})

When calculating effective degradability, rate constant of passage was assumed to be 0.02, 0.05 and 0.08 per h (Bhargava and Ørskov, 1987) so that the results could be extrapolated to other ruminants that differ in rumen capacity.

Statistical analysis: All of the data were analyzed based on completely randomized design (CRD), by using software of SAS (1991) and means (obtained from three samples) were separated by Duncan's multiple range tests (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Chemical composition of tomato pomace was shown in Table 1. The CP content of tomato pomace was lower than those reported by Boucque and Fiems (1988) and Chumpawadee *et al*. (2007) but higher than those National Academy of Science (1983), Weiss *et al*. (1997),

Table1: Chemical composition of microwave treated tomato pomace on dry matter basis (%).

DM	CP	EE	CA	ADF	NDF	NFC
94.46	24.08	13.98	5.73	33.6	47.8	8.41

DM: Dry Matter, CP: Crude Protein, EE: Ether Extract, CA: Crude Ash, ADF: Acid Detergent Fiber, NDF: Neutral Detergent Fiber, NFC: Non Fiber Carbohydrate

Table 2: Ruminal protein degradation (%) of microwave treated and untreated tomato pomace at different incubation times (h)

Incubation time (h)	untreated	microwave treated (2.5 min)	microwave treated (5 min)	p-value	S.E.M
0	15.20[a]	7.05[b]	4.57[b]	0.0095	1.05
4	30.20[a]	19.75[b]	15.67[c]	0.0008	1.25
8	39.60[a]	26.24[b]	27.37[c]	0.0001	1.54
12	53.60[a]	49.48[a]	40.33[b]	0.0018	1.11
16	56.65[a]	55.23[a]	53.35[b]	0.1163	1.12
24	59.87	58.45	54.95	0.1163	0.98
36	63.95[a]	63.11[a]	61.24[b]	0.0900	1.42
48	68.55	67.65	66.98	0.2000	1.78
72	74.55	73.35	73.21	0.1911	1.98

Means in the same row with different letters (a,b,c) differ ($p<0.05$)

Table 3: Ruminal protein degradation parameters and effective degradability of microwave treated and untreated tomato pomace

Items	Untreated	Microwave treated (2.5 min)	Microwave treated (5 min)	p-value	SEM
a (%)	13.20[a]	6.45[b]	4.08[c]	0.05	0.521
b (%)	51.26[b]	56.75[a]	58.94[a]	0.05	1.210
a+b (%)	64.46	63.20	63.02	0.12	0.841
c (h^{-1})	0.082	0.091	0.109	0.25	0.005
ERDP (%) Out flow rate 0.02 h^{-1}	54.96	53.62	53.17	0.81	0.035
ERDP (%) Out flow rate 0.05 h^{-1}	45.21	44.12	44.30	0.88	0.005
ERDP (%) Out flow rate 0.08 h^{-1}	40.11	40.27	41.13	0.48	0.047

Means in the same row with different letters (a,b,c) differ ($p<0.05$); *a*, washout fraction as measured by washing loss from nylon bags; *b*, potentially degradable fraction; *c*, rate of degradation of fraction b (h^{-1}). ERDP: effective rumen degradability of protein

Ayhan and Aktan (2004), Del Valle *et al*. (2006), Denek and Can (2006), Aghajanzadeh-Golshani *et al*. (2010) and Chumpawadee and Pimpa (2008) and in line with Besharati *et al*. (2008) and Taghizadeh *et al*. (2008). The wide range of variation in CP of by-products can be due to different original materials, growing conditions (geographic, seasonal variations, climatic conditions and soil characteristics), extent of foreign materials, impurities and different processing and measuring methods. Different chemical composition leads to different nutritive value, because chemical composition is one of the most important indices of nutritive value of feeds (Maheri-Sis *et al*., 2008; Aghajanzadeh-Golshani *et al*. 2010).

Ruminal protein degradation of microwave treated and untreated tomato pomace at different incubation times were shown in Table 2. Crude protein degradability in most of incubation times was significantly affected by the microwave irradiation. Microwave treating resulted in decreasing ruminal protein degradation, especially in early incubation times (early 12h). Increasing microwave irradiation times (5 min vs. 2.5 min) had higher effect on reducing protein solubility and subsequent degradability. This result is in line with findings of Sadeghi and Shawrang, (2006a, b) and Sadeghi and Shawrang, (2008). They are reported that increasing the microwave irradiation time lead to decreased the soluble fraction of protein (degradation at 0h incubation), presumably due to its heating effect on soluble proteins. These researchers concluded that, the decrease in CP degradation increased as microwave irradiation time increased. Previously Hoffman *et al*., (1999) indicated that microwave-drying decreases ruminal CP degradation of silages.

Effect of microwave irradiation on ruminal protein degradation parameters of tomato pomace were presented in Table 2. Increasing the microwave irradiation time decreased the *a* fraction and increased the *b* fraction of CP ($p<0.05$). The degradation rate of the *a* fraction decreased with increases in irradiation time. Although microwave irradiation lead to increase in degradation rate of the *b* fraction, but microwave irradiation time can not affect degradation rate of *b* fraction. The results were in line with previous reports (Sadeghi and Shawrang, 2006a, b; Sadeghi and Shawrang, 2008; Salamatdoust Nobar *et al*., 2010). Sadeghi and Shawrang (2006a) stated that the mechanisms behind the protection of protein against ruminal degradation in heat-treated feeds are complex. However, it is likely that chemical reactions (such as Millard reaction) occurring during heat processing are responsible for the reduction in ruminal degradation. These reactions will probably make the feedstuffs proteins more resistant against degradation in the rumen. Zhao *et al*. (2007) Suggested that, although microwave treatment causes less thermal damage to the test material than general heating methods such as hot water heating, it causes biochemical reactions and changes the molecular conformation of starch and protein, texture and physicochemical properties, such as the solubility and gelatin temperature of food products. Murray and Gellman (2007) expounded that microwave irradiation has been successfully applied to an ever-increasing number of organic reactions and synthesis of b-peptides and oligomers of b-amino acids (b-aa), could be enhanced by microwave irradiation.

As it is shown in Table 3, there were not differences between treatments in ERDP. It is predictable that when ($a+b$) and c were not significantly difference between treatments, ERDP was not affected significantly. However this result was not supported by findings of Sadeghi and Shawrang, (2006a, b) and Sadeghi and Shawrang, (2008) on canola meal, corn grain and barley grain. The difference between results of current study and above mentioned researches can be due to different protein nature of experimental feedstuffs, irradiation times and irradiation power.

CONCLUSION

Results of current study indicated that microwave irradiation resulted in decreasing soluble fraction and increase in potentially degradable fraction of tomato pomace protein. Increasing irradiation time led to further decrease in protein solubility. However microwave treatment can not improved effective rumen degradability of tomato pomace. It seems that effect of microwave treatment on rumen protein degradability was related to irradiation time (and /or power) and nature of proteins in different feedstuff. So that it is recommended that in further studies irradiation times considered more than 5 minutes and intestinal digestibility should be studied.

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