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*Short Communication*

# Ruminal dry matter degradability of treated soybean meal as source of escape protein

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**In order to study rumen degradation of dry matter for treating soybean meal with black liquor as source of xylose and microwave radiation, an experiment in nylon bag technique was performed. Samples for treating soybean meal for 0, 2, 4, 8, 16, 24 and 48 h in the rumen of three Taleshi male cows were incubated. Soybean meal samples treated with black liquor and microwave radiation decreased water soluble protein fraction (a) and increased slowly protein degradation fraction (b); and treatment with 6% black liquor and 4 min microwave radiation have suitable protection against the soybean meal dry matter degradability.**

**Key words:** Soybean meal, black liquor, microwave radiation, nylon bags.

## INTRODUCTION

Today, to provide protein requirements of lactating cows and fattening calves with high growth rate, using of slowly degradable protein in the rumen, but digestible in the small intestine is essential. Decreased degradability of soybean meal crude protein with different methods like thermal treating method (Van Soest, 1994; Waltz and Stern, 1989) including non-enzymatic browning for protection of feed protein degradability in rumen was performed. Proteins and carbohydrates at suitable temperature cause non-enzymatic browning reactions. Briefly, microwave energy penetrates a food or feed material and produces a volumetrically distributed heat source. This due to molecular friction, which results from dipolar rotation of polar solvents and from conductive migration of dissolved ions. The dipolar rotation is caused by variations of the electrical and magnetic fields in the organic components (Alton, 1998). Water, the major constituent of most food and feed products, is the main source for microwave interactions due to its dipolar nature. Black liquor is the spent cooking liquor from the kraft process when digesting pulpwood into paper pulp removing lignin, hemicelluloses and other extractives from the wood to free the cellulose fibers (Steniu, 2000).

The black liquor is an aqueous solution of lignin residues, hemicellulose, and the inorganic chemicals used in the process. The black liquor contains more than half of the energy content of the wood fed into the digester. It is normally concentrated to 65 - 80% by multi-effect evaporators and burned in a recovery boiler to produce energy and to recover the cooking chemicals. Hemicellulose is any of several heteropolymers (matrix polysaccharides), such as xylose; and xylose is a reducing sugar and could stimulate Maillard reaction. The Maillard reaction is a form of nonenzymatic browning similar to caramelization. It results from a chemical reaction between an amino acid and a reducing sugar, usually requiring heat. High temperature, intermediate moisture levels, and alkaline conditions all promote the Maillard reaction. Heat is generated throughout the material, leading to faster heating rates and shorter processing times compared to conventional heating, where heat is usually transferred from the surface to the interior (Fakhouri, and Ramaswamy, 1993). Other advantages include space savings and energy efficiency, since most of the electro-magnetic energy is converted into heat (Mermelstein, 1997). The purposes of this study were to evaluate effects of black liquor as source of xylose and microwave irradiation for stimulating Maillard reaction and for the evaluation of ruminal DM degradation of treated soybean meal.

**Abbreviations: SBM,** Soyabean meal; **DM,** dry matter.

**Table 1.** The rumen degradation characteristics of dry matter of treated soybean meal.

| Parameter | Microwave time | Black liquor level | Degradation characteristics (g/kg) | | | | Effective degradability of DM Pe (g/kg) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | a | b | a+b | c | **0.02 h$^{-1}$** | **0.05 h$^{-1}$** | **0.08 h$^{-1}$** |
| Control | --- | --- | 15.13$^{a}$ | 76.65$^{c}$ | 91.78$^{ab}$ | 0.092 | 78.37$^{ab}$ | 65.17 | 56.50 |
| SBM | 3 min | 3% | 12.96$^{b}$ | 80.02$^{ab}$ | 92.`99$^{a}$ | 0.098 | 79.57$^{a}$ | 66.07 | 57.17 |
| | | 6% | 11.36$^{b}$ | 79.50$^{b}$ | 90.87$^{ab}$ | 0.098 | 77.33$^{ab}$ | 63.97 | 55.10 |
| SBM | 4 min | 3% | 12.27$^{b}$ | 78.73$^{b}$ | 91$^{ab}$ | 0.105 | 78.20$^{ab}$ | 65.43 | 56.77 |
| | | 6% | 9.35$^{cd}$ | 83.75$^{a}$ | 93.1$^{a}$ | 0.098 | 78.67$^{ab}$ | 64.57 | 55.20 |
| SBM | 5 min | 3% | 12.49$^{b}$ | 77.99$^{b}$ | 90.48$^{b}$ | 0.096 | 76.83$^{b}$ | 63.57 | 54.80 |
| | | 6% | 10.68$^{c}$ | 80.53$^{ab}$ | 91.21$^{ab}$ | 0.096 | 77.00$^{b}$ | 63.37 | 54.30 |
| SEM | | | 1.099 | 1.5065 | 1.8202 | 1.4446 | 1.7666 | 1.9194 | 1.1501 |
| P value | | | <0.0001 | <0.0001 | 0.0310 | 0.5421 | 0.0238 | 0.5253 | 0.5904 |

## MATERIALS AND METHODS

### Sample preparation and treatment

The SBM samples (soybean meal imported from Brazil) were obtained from commercial sources in Iran. SBM was treated with 3 and 6% black liquor, with 20% additional water to solvent-extracted soybean meal and heated at a power of 900W for 3, 4 and 5 min via microwave irradiation. The mixture was then cooled to room temperature and air dried to approximately 10% moisture. The remainder of each sample was ground to pass a 2 mm screen for the ruminal *in situ* study and preserved as described earlier.

### Animal and diets

Three ruminal cannulated Iranian Taleshi native male cows weighing approximately 450 kg were placed in individual 4.2×2.8 m pens with concrete floors that were cleaned regularly. Cows were fed with 10 kg dry matter, a total mixed ratio containing concentrate and alfalfa hay diets twice daily at 09:00 and 16:00 h.

Untreated SBM: SBM + 3 min irradiation + 3% black liquor; SBM + 3 min irradiation + 6% black liquor; SBM + 4 min irradiation + 3% black liquor; SBM + 4 min irradiation + 6% black liquor; SBM + 5 min irradiation + 3% black liquor; SBM + 5 min irradiation + 6% black liquor

### *In situ* evaluation of dry matter

Nylon bag technique was used to measure disappearance in the rumen of untreated and treated SBM. Nylon bags (45 µm pore size; 10×15cm bag size) containing 5g of SBM samples were incubated in the rumen of each cow. The experiment was performed in a completely randomized design with three treatments and sex replications for each animal. Two bags of each type of treated SBM were removed after 2, 4, 8, 16, 24 and 48h of incubation in the rumen. Then, individual bags with contents were washed in running tap water until the bags were free of rumen content. To reach constant weight, bags were dried at 60°C for 48h. The solubility or washing loss was determined by soaking samples of each material in water at 37–40°C for 1h followed by the washing procedure above. Digestion kinetics of CP was determined according to the equation of Ørskov and McDonald (1979):

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

Where, p is the amount degraded at a time; a is the rapidly soluble fraction (g/kg); b is the potentially degradable fraction (g/kg); c is the constant rate of disappearance of b and t is the time of incubation (h). The effective rumen degradability of CP was estimated using the equation of Ørskov and McDonald (1979):

### Statistical analysis

Data were analyzed in a complete randomized design using the GLM procedure of SAS version 8.2 (SAS Inst. Inc., Cary, NC). For statistical analysis of data, Neway and SAS software package was used. After analysis of variance, least squares means of each sample by the Tukey test were compared.

$$y_{ij} = \mu + t_i + \varepsilon_{ij}$$

Where, $y_{ij} =$ all dependent variable; $\mu =$ over all mean; $t_i =$ the fixed effect of oil levels ($i = 1,2,3$); $\varepsilon_{ij} =$ the random effect of residual.

## RESULTS AND DISCUSSION

The rumen degradation characteristics of dry matter are presented in Table 1. Washing loss or (a) fraction was affected by treating with microwave irradiation and black liquor levels, significantly from 15.13% in control group and reaching 9.33-12.96 range in experimental treatments. All levels of black liquor with all the time of irradiation could effectively reduce (a) fraction, but 4 min heating and 6% of black liquor compared to others is better. The treating effect on the (b) fraction or slow degradation of DM was significant and 4 min microwave heating with 6% of black liquor was significantly increased compared to the control group, which from 76.65 got to 83.75%. Other experimental treatments compared to the control group were significantly higher, and results showed that Maillard reaction was done and this condition helps to increase resistance of SBM to degradation. Decrease of (a) fraction and increase of (b) fraction influence a+b; and treated group of 3 min irradiation with 3% of black liquor and 6 min irradiation with 6% of black liquor (92.99 and 93.1, respectively) were numerically higher compared to other treatments. The values for control group degradation are approximately similar to that reported by Deniz and Tuncer (1995) and similar to the results obtained in earlier studies (Mir et al., 1984; Windschitl and Stern, 1988; Harstad and Prestlokken, 2000). All discrepancies reported in *in situ* disappearance values can be attributed to varietals differences in the meal incubated, *in situ* technique, basal diet or variation to the extent of microbial contamination of the incubated samples (Freer and Dove, 1984; Nocek, 1988). Results show that effective degradation of DM in 0.02 $h^{-1}$ only in group treated with 3 min irradiation and 3% of black liquor significantly was higher compared to the control group and other treatment; but approximately they are in the same range (77-79%) and this condition is related to uniformity and small particle size of SBM. Moisture is necessary for non-enzymatic browning reactions to occur because water serves as the medium through which reactants interact. However, excessive moisture content in reaction mixtures can slow the rate of non-enzymatic browning through simple dilution of reactants and, because a molecule of water is produced for each amino sugar formed. In conclusion, non-enzymatic browning reduced treated soybean proteins degradability. Results suggest that non enzymatic reaction may be useful for increasing the amount of SBM which escapes ruminal degradation. Other chemical methods of protecting proteins from ruminal degradation include application of formaldehyde (Faichney, 1971; Madsen, 1982; Reis, and Tunks, 1969), tannins (Driedger and Hat-field, 1972; Thomas 1979 and divalent cations such as zinc (Britton and Klopfenstein, 1986).

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