

***In situ* evaluation of ruminal degradability and intestinal digestibility of extruded soybeans**

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ABSTRACT: Two ruminally cannulated Jersey cows were used to determine *in situ* ruminal degradability and intestinal digestibility of raw (E0) soybeans and soybeans extruded at 145°C (E145), 155°C (E155) and 165°C (E165). The degradation of dry matter (DM) and crude protein (CP) was estimated using nylon bags suspended in rumen for 2, 4, 8, 12, 16, 24 and 48 h calculating the effective ruminal degradabilities (ERD) for an assumed outflow rate of 0.06/h. Four extra sample bags were incubated for 12 h to determine CP digestibility using the mobile nylon bag method. Cows were fed a ration of 30% concentrate and 70% meadow hay. Soybean extrusion at all temperatures decreased the effective protein degradability significantly ($P < 0.05$). Soybean processing at the highest heat input (E165) decreased ERD (44.0%) in the rumen significantly while the values for E145 and E155 were similar, 50.8 and 50.3% respectively. Soybean extrusion at all tested temperatures was followed by a significant ($P < 0.05$) reduction in the proportion of the rapidly degraded DM and CP fractions a, and both the increased proportions and reduced breakdown rate (c) of the fraction b. The intestinal protein digestibility values of the rumen-undegraded protein of extruded soybean were somewhat higher, however the observed differences between treatments were not significant ($P < 0.05$). Estimated intestinal digestibilities were 87.2, 89.7, 92.0 and 92.6% for E0, E145, E155 and E165, respectively.

Keywords: extrusion; soybean; protein; ruminal degradability; intestinal digestibility

The protein evaluation systems assume that protein requirements for ruminants are met from microbial protein and undegraded dietary protein (UDP) digested in the small intestine. To achieve maximum productivity of high-producing or rapidly growing ruminants may require better quality protein than provided by rumen microorganisms (Waltz and Stern, 1989). The requirement of UDP increases with the performance of the animal. This protein can be supplied by reducing the ruminal degradation and thus increasing the amount of protein digested postruminally (Mustafa et al., 2000).

Full-fat soybean, which contains 40% CP and 17% of fat, is interesting as a source of protein and energy in rations for cows in the first part of lactation, but its protein is highly degradable in rumen (Grummer et al., 1994). Soybean protein is rich in lysine, methionine, valine and isoleucine constitut-

ing the first, second and third amino acid limiting milk production. For many years different physical and chemical methods have been researched to reduce rumen protein degradability and to increase the amino acid supply to the intestine. Heat treatments seem to be more effective than chemical ones protecting protein from ruminal degradation, some chemical method might have detrimental effects on digestibility of amino acids in the intestine (Mustafa et al., 2000). Extrusion (Benchaar and Moncoulon, 1993; Stern et al., 1985) and roasting (Lykos and Varga, 1995) caused a higher flow of total amino acids to the intestine while minimizing the activity of antinutritional factors (Aldrich et al., 1997). Improvements of milk yield in response to heat-treated soybeans were obtained by Schingoethe et al. (1996) and Kim et al. (1993), but other studies (Driver et al., 1990) did not show any improvement.

The objective of the study was to predict the effect of the extrusion temperature of whole soybean on degradation of DM and CP in nylon bag in the rumen and on CP digestibility in the intestines. We hypothesized that there is an optimal temperature of extrusion at which protein ruminal degradability is low and postruminal digestibility is maximized.

MATERIAL AND METHODS

Animals and feedstuffs

Two non-lactating Jersey cows, weighing 435 kg, fitted with cannulas in the rumen and in the proximal duodenum were kept in individual pens and fed a standard diet (14% CP, 25% CF) consisting of 30% concentrate (containing 50% barley, 18% wheat middlings, 10% wheat, 20% soybean meal and 2% vitamin and mineral mixture) and 70% meadow hay. Cows were given 50% of their assigned diet at 7.00 and 50% at 15.00 daily and had free access to water. The mean daily intake of dry matter was 8 kg.

Extrusion

Whole home-grown soybeans of (Polish var. *Nowiko*) were extruded in a commercial one-screw extruder (INSTA PRO 1500) at temperatures 145°C (E145), 155°C (E155) or 165°C (E165). They were ground through a 1.5-mm screen before their use in the following experiment.

In situ study

Quadruplicate nylon bags (2 bags for one cow) were filled with 5 g extruded soybeans and incubated for 0, 2, 4, 8, 12, 16, 24 and 48 hrs in the rumen. Four extra sample bags were incubated for 12 h to determine protein and CP digestibility using the mobile nylon bag method. The bags (200 × 90 mm, pore size 45 µm) were attached to a semi-rigid stalk and in turn they were attached to a swivel-connector inside the rumen fistula cap. Bags were placed simultaneously in the rumen just before the animals were offered the first meal in the morning (7.00 a.m.). After removal from the rumen, bags were washed with cold water and stored frozen. After defrosted, bags were washed, and then the bags were dried at 50°C for 48 h and

weighed. Zero-hour bags were not incubated but only washed.

Mobile bag technique

Four mobile bags per treatment were filled with one gram samples of the residue after 12 h ruminal incubation were inserted into mobile bags (80 × 25 mm, pore size 9 µm). The bags were incubated for 2 hours at 39°C in a pepsin-HCl solution (100 mg pepsin – 1 : 10 000/l of 0.004 mol/l HCl solution, pH 2.4) as recommended by Hvelplund et al. (1992) and inserted into the intestine via the duodenal fistula approximately one hour after feeding (8 bags per cow per 1 hour). The bags were recovered in the faeces within 26 hours. Bags not recovered within 26 h were discarded. After recovery, the bags were frozen, then washed, dried and weighed as described previously. The digestibility of undegradable protein was calculated as the amount of N lost from the bag during passage through the intestine divided by the amount of N in the bag before incubation.

Chemical analysis

The dry matter content was determined in all feedstuffs and nylon bag residues at 50°C for 48 h. Nitrogen was determined using Macro-Kjeldahl N analyser (AOAC, 1990). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined according to the method of Van Soest et al. (1991). Standard methods were also used to determine ash and fat (AOAC, 1990).

Calculations and statistical analysis

The percentage of DM and CP disappearing at each incubation time was calculated from the respective amounts remaining after incubation in the rumen. Data were fitted to a non-linear regression equation:

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

where: *a* = the rapidly soluble fraction

b = the potentially degradable fraction which disappears at a constant rate

c = per unit time (Ørskov and McDonald, 1979)

The three constants were then used to calculate the effective ruminal degradability (ERD) by the equation $ERD = a + [(b \times c)/(c + k)]$ assuming a fractional outflow rate (k) of 0.06/h.

Intestinal digestibility was estimated for 12 h of rumen incubation as follows: ruminally undegraded CP – residue after intestinal incubation/ruminally undegraded CP. Total tract digestibility was calculated for this same time as the sum of the CP ruminally degraded in 12 hours and the intestinally digested CP.

The results obtained for dry matter and protein degradability in the rumen and intestinal digestibility of UDP were subjected to analysis of variance. Significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

The increase in the extrusion temperature slightly decreased the ether extract content (Table 1), this is in agreement with the results of Benchaar et al. (1994). Soybean extrusion at all tested three temperatures was followed by a significant ($P < 0.05$) reduction in the proportion of the rapidly degraded DM and CP fractions a , and both the increased proportions and reduced breakdown rate (c) of the fraction b (Table 2). The decrease in soluble fractions by heat treatment is in agreement with others (Chouinard et al., 1997; Cros et al., 1991). Van Soest (1987) reported that rapidly degradable true protein fractions denatured at lower heat inputs and became intermediately or slowly degradable fractions based on the level of heat inputs while slowly degradable protein fractions responded at higher heat temperature and usually became unavailable. Soluble CP fraction (50.76%), degradation rate

of fraction b (0.1767/h) and effective protein degradability (83.1%) were highest for raw untreated soybeans (E0). Various sources (INRA, 2001; NRC, 2001) state that 75–80% of the protein is degraded in the rumen which limits its inclusion in diets for high-yielding ruminants. Soybean extrusion at 145, 155 and 165°C reduced EPD significantly by 33.1, 32.9 and 37.6%, respectively. Extrusion at 165°C reduced EPD more significantly than extrusion at 145 and 155°C, however no significant ($P < 0.05$) differences were observed between E145 and E155. The highest heat input (extrusion at 165°C) may penetrate the soybean, denature protein or form protein-carbohydrate complexes and could be more effective in altering and reducing nylon bag ruminal disappearance. According to Mir et al. (1984) soybean protein is less readily denatured by heat than rapeseed protein. Van Soest (1987) suggested that an optimum heat input depends on many factors: moisture content, carbohydrate content and composition, protein content, the presence of sulphite and therefore optimum parameters of heat treatment vary from one dietary protein to another. Aldrich et al. (1995) observed when the temperature of extrusion was increased (104, 140 and 160°C), the degradable fraction measured in *in situ* studies decreased (45.7, 36.7 and 30.1% respectively). Our studies indicate that the highest temperature of extrusion (165°C) was effective in the protection of soybean protein against microbial degradation in the rumen however no optimal temperature of extrusion was determined. Higher heat input than in our study may warrant further investigation.

Orias et al. (2002) found that soybean extrusion at a high temperature (160°C) did not affect bacterial synthesis because it did not negatively

Table 1. Chemical composition of extruded soybeans (% of DM)

Item	Soybeans			
	E0	E145	E155	E165
Dry matter	88.7	90.5	91.3	90.8
Crude protein	37.2	36.4	36.9	36.8
Ether extract	19.0	19.1	18.4	18.0
Ash	6.1	5.1	5.2	5.2
Crude fibre	7.2	6.8	6.4	6.0
Neutral Detergent Fibre	30.4	29.6	27.6	27.8
Acid Detergent Fibre	21.0	20.4	19.3	19.2

Table 2. Degradation parameters and effective degradability of dry matter and crude protein of extruded soybeans

Item	Soybeans			
	E0	E145	E155	E165
Dry matter				
<i>a</i> (%)	40.05*	13.54**	12.92**	11.38**
<i>b</i> (%)	54.32*	84.20**	82.74**	91.23**
<i>c</i> (h)	0.1610*	0.0534**	0.0576**	0.0467**
ERD (%)	79.62*	53.13**	53.39**	51.26**
Crude protein				
<i>a</i> (%)	50.76*	11.56**	11.18**	6.51***
<i>b</i> (%)	43.32*	83.47**	83.38**	96.08***
<i>c</i> (h)	0.1767*	0.0534**	0.0531**	0.0467***
ERD (%)	83.10*	50.80**	50.26**	44.03***

*, **, ***values with different superscripts are significant $P < 0.05$

effective ruminal degradabilities (ERD) = $a + [(b \times c)/(c + k)]$ with $k = 0.06/h$

a = rapidly soluble fraction; *b* = potentially degradable fraction; *c* = fractional degradation rate of fraction *b*

affect organic matter digestion or N availability for the rumen microbes. Heating soybean at that temperature increased amino acid digestion in the small intestine (except methionine and glycine). Similarly to our findings, Stern et al. (1985) showed that the heat treatment of soybeans by extrusion at 149°C decreased protein ruminal degradability compared to untreated soybeans while extrusion at a lower temperature (132°C) was not beneficial for decreasing protein rumen degradability. Aldrich et al. (1997) reported that soybean extrusion at 116, 138 and 160°C decreased rumen DM disappearances after 16 h incubation from 69.9, 54.9 to 56.3% respectively. However, Mir et al. (1984) observed an increase in effective protein degradability when soybeans were heated at 110°C for 2 h, suggesting that it may be due to the destruction of antinutritional factors. The results of the study also indicated that temperature is more important than the duration of heat treatment. Orias et al. (2002) concluded that extruding soybeans at 116 or 138°C may not be sufficient to protect soy proteins from ruminal degradation. Data from Faldet and Satter (1991) demonstrated lower values for the degradation kinetics of roasted soybean. Lykos and Varga (1995) observed no significant effect of roasting cracked soybean at 144°C and a significant decrease

in protein degradability of ground and roasted soybean at the same temperature.

Soybean heating over the optimum temperature may protect against microbial degradation in the rumen and also make protein indigestible in the intestine due to Maillard reaction between sugars and proteins. Lin and Kung (1999) suggested that overheating increased a possibility of forming insoluble melanoidins which are toxic both for microorganisms and for the host animal. In the present study the extrusion of whole soybeans decreased rumen disappearance but intestinal digestibility of undegraded protein was not affected by the temperature of heat treatment, suggesting that heat extrusion did not have a negative effect on intestinal digestibility (Table 3). The values of CP intestinal digestibility of extruded soybean were somewhat higher, however the observed differences between treatments were not significant ($P < 0.05$). Estimated intestinal digestibilities were 87.2, 89.7, 92.0 and 92.6% for E0, E145, E155 and E165, respectively. Heating the soybeans over the optimum temperature may protect against microbial activity in the rumen and also make protein indigestible in the intestine. Stern et al. (1985) and Aldrich et al. (1995) reported that the heat process increased intestinal protein digestibility by destruction of the

Table 3. *In situ* crude protein disappearance of extruded soybeans and intestinal digestibility of ruminally undegraded crude protein (values of 12 h of rumen incubation)

Item	Soybeans			
	E0	E145	E155	E165
Rumen disappearance RD (%)	71.07*	52.23**	54.34**	45.34***
Intestinal digestibility of RUP (%)	87.20	89.67	92.04	92.56
Total tract digestibility (%)	96.30	95.06	96.36	95.96

*, **, ***values with different superscripts are significant $P < 0.05$

total tract digestibility = $RD + (100 - RD) \times \text{intestinal digestibility of RUP}/100$

trypsin-inhibiting activity in beans. However, Orias et al. (2002) suggested that the trypsin inhibitor complex received little attention in ruminant nutrition because it was assumed to be largely degraded by rumen microbes.

Intestinal digestibility was in proportion to the protein concentration in nylon bags. These data indicate that having escaped the microbial attack protein in the reticulorumen was well digestible in the small intestine. Extrusion significantly decreased ($P < 0.05$) CP disappearance during ruminal incubation for 12 h, but the whole tract digestibility was not significantly affected.

Improvements of digestibility of CP and/or amino acids in the small intestine were reported for ruminants fed roasted (Tice et al., 1993) and extruded soybean (Stern et al., 1985; Aldrich et al., 1997). The percentage content observed in the present study for the total tract digestion of CP was high and similar to that obtained by Benchaar et al. (1994) and Cros et al. (1991) for extruded field beans and Arieli et al. (1989) for whole cottonseeds. The increasing temperature of Jet-Sploding of whole canola seeds from 116 to 177°C decreased effective protein degradability from 61.0 to 34.7% without reducing digestibility of undegradable protein in the intestine (Kennely and de Boer, 1986). McKinnon et al. (1995) studied the effect of heat-treated canola meal on intestinal digestibility. They found that heating to 145°C (30 min) decreased intestinal and total tract digestibility significantly however heating at 125°C lowered ruminal degradability without negative effects on intestinal digestibility. Lykos and Varga (1995) concluded that there occur reactions between proteins, resulting in protease-resistant cross-links between polypeptide chains that may or may not resist acid hydrolysis depending on the intensity and duration of heat treatment. The extrusion of soybean seed meals did not have

a positive effect on ruminal degradation and total tract digestibility and extreme heat treatment may decrease protein disappearance in the rumen together with decreasing intestinal availability of amino acids (Deacon et al., 1988). Benchaar and Moncoulon (1993) observed that the extrusion of lupine seeds at 195°C decreased rumen protein degradability (from 94.6 to 37.7%) without negative effects on intestinal digestibility of ruminal undegraded protein. Benchaar et al. (1994) reported that the extrusion of whole horse beans at 195°C decreased rumen protein disappearance from 90 to 58% and increased intestinal availability of ruminally undegraded dietary CP from 78 to 91%. Field beans extruded at 120°C decreased effective protein degradability from 89 to 70% without adverse effect on total crude protein digestibility (Cros et al., 1991). Lindberg et al. (1982) found that heating whole rapeseeds at 150 and 200°C decreased EPD by 18 and 52%, respectively, however heating at 100°C had no significant effect on ruminal protein degradability.

CONCLUSIONS

Whole soybean CP was effectively protected from degradation in the rumen by extrusion without negative effect on intestinal digestibility of UDP. The highest temperature of extrusion (165°C) caused a slight but significant decrease in effective protein degradability and elevated the supply of dietary digestible CP to the intestine.

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