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Short communication

Effects of non-enzymatic browning reaction intensity on *in vitro* ruminal protein degradation and intestinal protein digestion of soybean and cottonseed meals

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ABSTRACT

The effects of non-enzymatic browning reactions on *in vitro* ruminal gas production and *in vitro* ruminal and intestinal crude protein (CP) digestibilities of soybean (SBM) and cottonseed (CSM) meals were investigated. Non-enzymatically browned SBM and CSM samples were prepared using two xylose levels (10 or 30 g/kg dry matter), two heating lengths (30 or 60 min) and two heating temperatures (120 or 150 °C) for a total of one untreated (commercially solvent-extracted, Control) and eight treated samples for each protein source. The control SBM had higher (P<0.001) *in vitro* ruminal CP degradability values than the treated samples. Intestinal protein digestibility and total-tract CP digestibility of CSM and SBM were affected by the treatment (P<0.01). The results of the study indicate that not only ruminal CP degradability is reduced but also intestinal and total-tract CP digestibilities may be lowered depending on protein source and intensity of the non-enzymatic browning reaction.

1. Introduction

Determination of the metabolizable protein values of feeds for ruminants focuses on the quantity of absorbable amino acids supplied by the feeds to the small intestine. Accurate estimation of the quantity of rumen undegradable crude protein (CP) and its intestinal digestibility is crucial for metabolizable protein systems. Oilseeds are mostly processed to remove the oil for use as an edible fat. The defatted co-products, *e.g.*, soybean (SBM) and cottonseed (CSM) meals, are commonly used as protein supplements for farm animals.

Several techniques have been used to determine the ruminal degradation and whole-tract digestibility of CP of ruminant feeds. The techniques include *in vivo* and *in vitro* methods, the former necessitating the use of live intact or surgically modified animals. Calsamiglia and Stern (1995) developed a three-step *in situ–in vitro* procedure to estimate intestinal CP digestibility in ruminants. The technique was developed to closely simulate physiological conditions in the ruminants' digestive tract, be rapid, reliable and inexpensive, be applied to a wide variety of protein supplements and accurately reflect differences in protein digestion (Calsamiglia and Stern, 1995).

Abbreviations: ADF, acid detergent fibre expressed inclusive of residual ash; CP, crude protein; CSM, cottonseed meal; DM, dry matter; HL, heating length; HT, heating temperature; ICP, initial CP; IPD, intestinal protein digestibility; RCP, residual CP; RCPD, ruminal CP degradability; RUCP, rumen undegradable CP; TCPD, total-tract CP digestibility; TP, true protein; XYL, xylose levels.

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Table 1
Chemical composition (g/kg dry matter (DM) basis unless stated) of untreated (Control) and differently treated soybean (SBM) and cottonseed (CSM) meals.

Treatments	DM[a]	Ash	ADF	CP	True Protein	Gossypol	
						Total	Free
Soybean meal							
Control	925.2	70.1	107.3	519.7	459.6	NA	NA
T1 (120[b]–30[c]–10[d])	928.8	70.4	129.3	520.9	448.2	NA	NA
T2 (120–60–10)	931.7	70.8	148.2	519.2	471.1	NA	NA
T3 (120–30–30)	927.3	70.0	136.9	512.1	433.7	NA	NA
T4 (120–60–30)	930.9	70.4	142.0	513.3	434.4	NA	NA
T5 (150–30–10)	932.2	71.8	152.9	519.5	431.2	NA	NA
T6 (150–60–10)	931.6	71.8	189.8	516.5	465.7	NA	NA
T7 (150–30–30)	931.6	70.6	188.8	515.0	432.9	NA	NA
T8 (150–60–30)	933.9	71.0	177.4	514.7	446.7	NA	NA
Cottonseed meal							
Control	943.3	66.1	387.9	252.0	200.6	6.6	0.8
T1 (120–30–10)	937.2	65.1	375.0	233.1	173.8	5.7	0.5
T2 (120–60–10)	936.4	62.7	402.6	242.8	184.3	5.7	0.4
T3 (120–30–30)	933.9	61.5	402.9	229.6	175.7	7.0	0.6
T4 (120–60–30)	932.1	62.9	392.0	231.6	172.3	6.2	0.5
T5 (150–30–10)	932.7	64.7	387.3	237.8	183.4	5.6	0.4
T6 (150–60–10)	932.8	65.1	398.3	246.2	182.9	5.0	0.2
T7 (150–30–30)	931.8	63.6	403.5	225.0	190.8	5.6	0.5
T8 (150–60–30)	929.8	65.7	435.0	234.4	188.3	5.1	0.5

[a] ADF, acid detergent fibre expressed inclusive of residual ash; CP, crude protein; NA, not analyzed.
[b] Heating temperature (°C).
[c] Heating length (min).
[d] Xylose level (g/kg DM).

Heat processing is used commercially to reduce the degradation of protein supplements by ruminal microorganisms. This reduction occurs due to Maillard reactions between sugar residues and amino acids (Van Soest, 1982). Factors regulating rate of the Maillard reaction include type and concentration of reducing sugar (Spark, 1969; Hashiba, 1982; Can and Yilmaz, 2002) and temperature and duration of heating time (Cleale et al., 1987b; Can and Yilmaz, 2002). McNiven et al. (2002) reported a strong significant correlation between CP digestibilities estimated by mobile bag and *in vitro* procedures using grain and SBM samples, and concluded that the *in vitro* method could accurately estimate CP digestibility and was sensitive to the effects of heat treatment. However, data on ruminal and postruminal digestion of non-enzymatically browned protein sources is limited. Therefore, the objective of this study was to determine the effects of protecting SBM and CSM with non-enzymatic browning reactions including combinations of xylose level, heating length and heating temperature, on *in vitro* ruminal and intestinal CP digestibilities.

2. Materials and methods

2.1. Non-enzymatic browning procedure

Non-enzymatically browned samples of SBM and CSM with hulls were prepared using each of two xylose levels (XYL; 10 or 30 g/kg dry matter (DM)), heating lengths (HL; 30 or 60 min) and heating temperatures (HT; 120 or 150 °C) for a total of one untreated (commercially solvent-extracted, Control) and eight treated samples for each protein source. The chemical composition of the samples is presented in Table 1. Before the non-enzymatic browning reaction was induced, SBM and CSM samples were ground through a 2-mm screen. Following the addition of appropriate quantities of xylose, distilled water was added so that each sample contained DM at 700 g/kg. The heated samples were obtained by placing material corresponding to 200 g DM in 22-cm diameter aluminum pans, covering the pans with aluminum foil and heating them in a forced-air oven at temperatures of 120 or 150 °C for either 30 or 60 min. The samples were then cooled to room temperature and air-dried for 72 h (Can and Yilmaz, 2002).

2.2. Enzymatic procedure for ruminal and intestinal crude protein digestion

Before enzymatic treatment, samples were reground through a 1-mm screen (Model M20; IKA, Staufen, Germany). The three-step enzymatic procedure followed Calsamiglia and Stern (1995) except for the first step that simulates rumen incubation which was done according to Irshaid (2007) and Irshaid and Suedekum (2007), who replaced the original *in situ* rumen degradation step with a standardized *Streptomyces griseus* protease incubation. The true protein (TP) contents of all samples were determined using trichloroacetic acid (1000 g/l) as precipitating agent (Licitra et al., 1996). Based on the TP concentration of the samples, addition of a *S. griseus* protease solution was adjusted to the ratio of 41 U/g TP (Licitra et al., 1998, 1999) for ruminal protein degradation. Samples (2.5 g) were accurately weighed into 500-ml Erlenmeyer flasks and 200 ml of borate–phosphate buffer (pH 6.7–6.8) were added. After adding the required amount of protease solution, flasks