Estimation of intestinal protein digestibility of rapeseed and soybean products using a three-step enzymatic in vitro procedure

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ABSTRACT

This study included 33 samples with main focus on unprotected or rumen protected rapeseed and soybean feedstuffs, which were analysed using an enzymatic *in vitro* procedure (EIVP) in order to determine intestinal crude protein (CP) digestibility (IPD) of ruminally undegraded CP (RUP). The EIVP involved the sequential digestion of samples with a protease from *Streptomyces griseus*, pepsin-HCl and pancreatin. The concentration of *S. griseus* protease was related to the true protein content of the feed sample. Briefly, the EIVP started with determination of true protein. Feeds were incubated for 18 h in a buffer solution at a constant ratio (14 U/g) of *S. griseus* protease activity to feed true protein. The dried residues were incubated in pepsin-HCl solution for 1 h and residues from this step were incubated in pancreatin solution for 24 h. Generally, chemically treated soybean and rapeseed products revealed approximately 20% higher IPD values than physically treated feedstuffs. Moreover, results appeared to have lower IPD dimensions than literature-data of previous studies. In addition, correlation analysis of IPD in relation to different nutrient values revealed a negative correlation between acid detergent fibre and IPD, as well as a positive correlation between crude protein, true protein and IPD. To sum up, the EIVP seems to be a reliable, simple laboratory method to estimate IPD of RUP in concentrate feeds. However, future studies may be constricted since sufficient reference values, e.g. *in vivo* data is missing.

Keywords: Rumen, protein, rapeseed meal, soybean meal, enzymatic *in vitro* procedure

INTRODUCTION

Crude protein (CP) values of feeds do not supply precise information about the protein that may actually be digested in the small intestine by ruminants. The CP reaching the small intestine consists of both, the ruminally synthesized microbial CP as well as the feed CP that escaped ruminal degradation. Several techniques are available to determine ruminal degradation and whole-tract digestibility of CP. These techniques include *in vivo* and *in vitro* methods, and differ highly in complexity, cost and effort. Calsamiglia and Stern (1995) developed a three-step in *situ-in vitro* procedure (ISIVP) to estimate intestinal CP digestibility (IPD) by simulating physiological conditions in the ruminants` digestive tract. The method is supposed to be rapid, reliable and inexpensive, can be applied to a wide variety of protein supplements and accurately reflects differences in protein digestion (Calsamiglia and Stern, 1995). However, the procedure includes ruminal incubation of samples which may be regarded as an additional error source and still might be unable to compete with simple laboratory methods. Subsequently, Irshaid (2007) refined the procedure and developed an enzymatic *in vitro* procedure (EIVP) by replacing the rumen incubation step of Calsamiglia and Stern (1995) with an enzymatic treatment using a protease from *Streptomyces griseus* to mimic ruminal degradation of CP. Although values for ruminally undegraded dietary protein (RUP) are existing for a large number of feeds, there are remarkable gaps in regard to reliable data, in particular for protein supplements like solvent-extracted oilseed meals, especially rapeseed and soybean commodities, which are considered to be an important source for high-quality protein to all farm animal species. For this reason, the main objective of this study was to evaluate IPD of RUP of several protein supplements which were predominantly characterised as protected from ruminal degradation through specific technical treatments, via the EIVP (Irshaid, 2007). The second aim of this study was to evaluate relationships between calculated IPD values and analysed chemical variables of feedstuffs.

MATERIALS AND METHODS

Feedstuffs

This study included 33 samples, with a main focus laid on rapeseed meal and soybean meal feedstuffs. Twenty-three samples were protected from rumen degradation either by a physical, namely thermal treatment (13 samples) or by chemical treatment (10 samples). Chemical treatments included formaldehyde (4 samples), xylose (5 samples) or polyurea-formaldehyde (1 sample) additions, in order to decrease ruminal CP degradation. Further, 5 samples were specifically assembled for experimental purposes, while the remaining samples were commercially purchased. Moreover, this study included samples of wheat grain, lupine and solvent-extracted sunflower meal. Crude protein values of the feedstuffs in their entirety ranged between approximately 140 to 500 g/kg dry matter (DM).

Enzymatic in vitro procedure

Before enzymatic treatment, samples were ground through a 1-mm screen (Model M 20; IKA, Staufen, Germany). The three-step enzymatic procedure (ISIVP) followed Calsamiglia and Stern (1995) except for the first step that stimulates rumen incubation, which was done according to Irshaid (2007) and Irshaid and Südekum (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 true protein (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 were incubated in a shaking water bath at 39°C for 18 h. Following, the content was filtered with the aid of a mild vacuum through a filter bag (38 µm pore size). Residues were washed with 1.25 l deionized water and dried in a forced-air oven at 55°C for 48 h.

Four replicates of each feed sample residue were weighed into 50-ml centrifugation tubes in an amount corresponding to 15 mg N for intestinal protein digestion. Subsequently, 10 mL of a 0.1 N HCl solution (pH 1.9) containing 1 g/l of pepsin were added to each tube and tubes were incubated at 38°C for 1 h in a shaking water bath. After incubation, pH was neutralized with 0.5 ml of 1 N NaOH; then 13.5 ml of a phosphate buffer (pH 7.8) containing 37.5 mg of pancreatin were added to each tube which was then vortexed and incubated at 38°C. Immediately after a 24 h incubation, 3 ml of trichloroacetic acid solution (1000 g/l) were added to each tube to stop enzymatic action and precipitate undigested protein.

Statistical analysis and calculations

Intestinal protein digestibility (IPD; g/kg CP) was estimated as:

IPD = (RUP - RCP)/ RUP x 1000;

where RUP, the rumen undegradable CP content (g) of the feed sample which was weighed into a 50-ml centrifugation tube and RCP, residual CP content (g) of the precipitate.

PROC CORR of SAS 9.2 (SAS® 2009) tested potential relations between calculated IPD values in relation to DM, ash, acid detergent fibre (ADF, expressed inclusive residual ash) and CP. Pearson's correlation coefficient was reported from PROC CORR as an indicator of the strength and the direction of these relationships. Relations between these variables and IPD were considered significant at $P < 0.05$.

RESULTS

For rapeseed products mean IPD values gathered at 648 g/kg CP and soybean feedstuffs exhibited an average value of 755 g/kg CP. An unprotected rapeseed meal ranged at 533 g/kg CP and a soybean-rapeseed meal mixture which included protein, labelled as protected through a physical heat-pressure treatment ranged at 619 g/kg CP. A formaldehyde-treated soybean revealed 880 g/kg CP and estimation for rapeseed meals resulted in 789 g/kg CP.. Unprotected rapeseed meals resulted in similar values than the treated samples (820 g/kg CP). Likewise mentionable results were observed for non-rapeseed-soybean meal samples. Chemically treated wheat grain resulted in IPD values of 840 g/kg CP and non-treated rapeseed hulls exhibited IPD values around 182 g/kg CP.

Analysis of correlation coefficients revealed a strong negative correlation between ADF values and IPD (r = -0.718, $P < 0.001$) as well as positive correlations between CP and IPD (r= 0.4535, P = 0.008) as well as TP and IPD (r = 0.46111, P = 0.0069).

DISCUSSION

Recently, Irshaid (2007) complemented the established three-step ISIVP by Calsamiglia and Stern (1995) and found evidence that IPD values estimated from the new procedure (EIVP) compared well with data derived from ISIVP (r^2 = 0.98, $P < 0.0001$) and data obtained by the mobile bag technique (r^2 = 0.66, $P < 0.0001$) by Hvelplund (1985) and Hvelplund et al. (1992). Similarly, present results are in agreement with previous studies which have been performed to evaluate IPD in concentrate feedstuffs (Gargallo et al., 2006; Stern et al., 1997; Woods et al., 2003). Nevertheless, it appears that findings of ISIVP and mobile bag technique result in higher IPD values than the ones determined in the present study. Since both procedures include ruminal incubation of the samples, higher variations of results are possible, mainly due to factors like animal characteristics, bag or temporal properties as well as other procedural aspects (Vanzant et al., 1998). Consequently, the EIVP could be more precise than established *in situ* methods since laboratory standardization may be easier to accomplish than diminishing individual animal effects.

In contrast to the present study, Irshaid (2007) did not observe any correlations between IPD and CP or TP. However, the negative correlation between IPD and ADF values has been observed by Irshaid (2007) as well. Lignocellulose, mainly composed of cellulose and lignin, is one of the main factors affecting energy and nutrient supply to ruminal microbes. Moreover, cell wall contents are a limiting factor for DM intake. This knowledge and the fact that ADF generally includes less available CP lead to the assumption that feeds having higher lignocellulose concentrations generally will have lower IPD values.

CONCLUSIONS

The comparison between experimentally determined results and literature data resulted in agreements as well as variations. In order to evaluate if absolute results are plausible and applicable, further research regarding IPD of RUP is required. However, the EIVP seems to be an adequately working method to estimate IPD of RUP in concentrate feeds and moreover can be utilized to help classifying different feedstuffs. Nevertheless, future studies may be hindered since sufficient reference values, e.g. *in vivo* data is completely missing.

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