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Replacing maize silage plus soybean meal with red clover silage plus wheat grain in diets of dairy cows: Modelling the utilizable crude protein at the duodenum, a precursor to metabolizable protein

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ABSTRACT

The aim was to evaluate the effect of replacing maize silage (MS) plus soybean meal with red clover silage (RCS) plus wheat grain in total mixed rations (TMR) on utilizable crude protein at the duodenum (uCP), and to evaluate the modified Hohenheim gas test (moHGT) for estimation of uCP supply in dairy cows. The TMR comprised forage and concentrates (0.75:0.25), and the forage portion comprised of MS and RCS. The RCS accounted for 0.15, 0.30, 0.45, or 0.60 of the TMR dry matter. The uCP of the TMR was calculated using the "standard German procedure" as the reference value (uCP_{GfE}) as the sum of ruminally undegraded feed crude protein (RUP_{GfE}) and ruminally synthesized microbial crude protein (MCP_{GfE}). For this, the RUP_{GfE} content was calculated from RUP content based on in situ ruminal degradation of crude protein, and the MCP_{GfE} was calculated based on the contents of crude protein, RUP and metabolizable energy. The moHGT was used to determine the in vitro uCP (uCP_{moHGT}) and was compared with uCP_{GfE}. The RUP_{GfE}, MCP_{GfE}, uCP_{GfE} and uCP_{moHGT} were estimated assuming passage rates out of the rumen of 2, 5 and 8%/h. At all assumed passage rates, replacing MS with RCS linearly decreased ($P < 0.001$) the RUP_{GfE}, MCP_{GfE} and uCP_{GfE} content of the TMR. The uCP_{moHGT} at passage rates of 5 and 8%/h was closely correlated with uCP_{GfE} ($R^2 = 0.94$, $P = 0.03$, and $R^2 = 0.95$, $P = 0.02$, respectively), whereas for a passage rate of 2%/h there was no significant correlation ($R^2 = 0.72$, $P = 0.15$). In conclusion, increasing the proportion of RCS plus wheat grain in the diet of dairy cows reduced the amount of uCP_{GfE} and this was caused more by a reduction in the RUP_{GfE} than in the MCP_{GfE} content. The moHGT has considerable potential as an accurate method for estimating the uCP of TMR, but further studies on standardization of the method and validation with a greater number of TMR samples are recommended.

Abbreviations: ADFom, acid detergent fibre expressed exclusive of residual ash; CA, crude ash; CP, crude protein; DM, dry matter; MCP, ruminally synthesized microbial CP; ME, metabolizable energy; moHGT, modified Hohenheim gas test; MS, maize silage; aNDFom, neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash; NPN, non-protein nitrogen; OM, organic matter; PD, purine derivates; RC, red clover; RCS, RC silage; RUP, ruminally undegraded feed CP; SBM, soybean meal; TMR, total mixed ration; uCP, utilizable CP at the duodenum

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1. Introduction

Red clover (*Trifolium pratense* L.; RC) is a forage legume that is widely used for silage (RCS) production in northern Europe and North America (Moorby et al., 2016) and has the potential to sustain milk production largely due to its high crude protein (CP) content. Compared to grass silage, RCS has high levels of soluble CP in the form of non-protein nitrogen (NPN) compounds and relatively low energy content (Dewhurst, 2013). In direct contrast, maize silage (MS) has a low CP content and is a significant source of soluble carbohydrates compared to grass silage and RCS, which provide rapidly fermentable energy to ruminal microbes; it can therefore complement the high NPN and low energy content of RCS (Moorby et al., 2016). It has been hypothesized that combining RCS and MS would maximize the supply of uCP (Westreicher-Kristen et al., 2017). However, only a few studies have been conducted based on mixtures of RCS and MS for dairy cows (Broderick et al., 2001; Dewhurst et al., 2010; Moorby et al., 2016; Schulz et al., 2018).

To evaluate the suitability of a feed, accurate characterization of the protein value is needed along with feeding trials. One key variable for the characterization of feed CP according to the Society of Nutrition Physiology in Germany (GfE, 2001) is the utilizable CP at the duodenum (uCP), which is the sum of ruminally undegraded feed CP (RUP) and ruminally synthesized microbial CP (MCP). In the German protein evaluation system (GfE, 2001) the uCP content of feedstuffs is calculated based on concentrations of RUP, CP and metabolizable energy (ME). The GfE equation is derived from results of in vivo experiments with duodenally fistulated dairy cows conducted at various feeding levels using mixed rations. The measurement of uCP in vivo is expensive, laborious and time-consuming. On the other hand, calculating the uCP based on the GfE equation (2001) requires measurement of the RUP content, which is normally calculated on the basis of in situ ruminal degradation of CP using rumen-fistulated animals; this makes the procedure inappropriate for routine analysis. In vitro techniques can quickly and cost effectively deliver information about feed values. In line with this, Steingass and Südekum (2013) proposed a modification of the Hohenheim gas test (moHGT; Menke and Steingass, 1988) for estimation of uCP in feedstuffs. This technique is based on the measurement of ammonia-N after incubation of feed samples with an incubation medium made from a mixture of buffer solution and rumen liquor. To date, the moHGT has been used to evaluate individual feeds for cattle like grass and legume forages, feed proteins and by-products. However, to our knowledge, no published study so far exits evaluating total mixed rations (TMR) for dairy cows.

Considering the importance of quantifying the optimal mixture of RCS and MS in TMR and the relevance of validating the moHGT for routine determination of uCP of TMR for dairy cows, this study aimed to (1) evaluate the effect of replacing MS plus soybean meal (SBM) with RCS plus wheat grain in TMR on uCP concentration calculated using the standard procedure for the German system and based on in situ measurement of RUP, and (2) validate in vitro uCP values with those of the standard procedure. It was hypothesized that (1) increasing levels of RCS plus wheat grain would reduce the uCP content in diets of dairy cows, and (2) the uCP content of TMR can be accurately estimated using the in vitro moHGT technique.

2. Materials and methods

2.1. Rations studied

The four TMR studied here were obtained from a feeding trial with dairy cows (Schulz et al., 2018). The sampled TMR had a constant forage to concentrate ratio (0.75:0.25), and the forage portion was comprised of MS and RCS. Maize silage was replaced by RCS with targeted proportions of RCS of 0.15 (diet RCS15), 0.30 (diet RCS30), 0.45 (diet RCS45), and 0.60 (diet RCS60) in the TMR on a dry matter (DM) basis. The concentrates were based on lupin seed, SBM, wheat grain and a mineral-vitamin premix. Diets were formulated to be similar in CP content. The analysis of proximal constituents and fibre fractions were performed following the official analytical methods in Germany (VDLUFA, 2007) and is described in Westreicher-Kristen et al. (2017). The CP fractions (Table 1) were calculated according to the Cornell Net Carbohydrate and Protein System (Sniffen et al., 1992; CNCPS) and were reported in Westreicher-Kristen et al. (2018).

2.2. Calculation of reference values of utilizable crude protein at the duodenum

The equation number 9 of the Society of Nutrition Physiology in Germany (GfE, 2001) was used to calculate the reference values of uCP (uCP_{GfE}) of the TMR as the sum of RUP_{GfE} and MCP_{GfE}:

$$RUP_{GfE}\ (g/kg\ DM) = 1.03 \times RUP$$

$$MCP_{GfE}\ (g/kg\ DM) = [11.93 - (6.82 \times (RUP/CP))] \times ME$$

where RUP was based on in situ ruminal degradation of CP of the diets (n = 4 for each TMR) and was published in Westreicher-Kristen et al. (2018). Assumed passage rates of 2, 5 and 8%/h were chosen to calculate RUP_{GfE}. The content of ME is in MJ/kg DM (Table 1) and was published by Westreicher-Kristen et al. (2017), and the content of CP is given in g/kg DM. Consequently, values at different passage rates were obtained for MCP_{GfE} and uCP_{GfE}. The modified Hohenheim gas test (moHGT) was used to measure the in vitro uCP (uCP_{moHGT}) of the TMR and results were published by Westreicher-Kristen et al. (2017). The uCP_{moHGT} were compared with uCP_{GfE}. The moHGT was carried out based on Menke and Steingass (1988), with modifications described by Steingass and Südekum (2013). Succinctly, samples of the four TMR were incubated in triplicate in four consecutive runs in an incubation medium made from a buffer solution and rumen liquor. The samples were incubated for 8 and 48 h, and uCP was calculated for each incubation time