Rumen fermentation and microbial yield of high- or low-protein diets containing ground soybean seeds or homemade rapeseed expellers evaluated with RUSITEC

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Guadagnin, M., Tagliapietra, F., Cattani, M., Schiavon, S., Worgan, H. J., Belanche, A., Newbold, C. J. and Bailoni, L. 2013. **Rumen fermentation and microbial yield of high- or low-protein diets containing ground soybean seeds or homemade rapeseed expellers evaluated with RUSITEC**. Can. J. Anim. Sci. **93**: 363–371. This experiment aimed to compare diets containing two crude protein (CP) concentrations [147 or 109 g kg^{-1} in dry matter (DM)] and two protein sources containing ground soybean seed (GSS) or rapeseed expeller (RSE). Diets were compared in terms of digestibility, volatile fatty acids (VFA) and ammonia concentrations, and N flows, using rumen simulation fermenters (RUSITEC). Home – made RSE (CP = 287 g kg^{-1} dry matter and ether extract = 199 g kg^{-1} DM) was produced using equipment adopted by small farms. Reduction of dietary CP content did not affect digestibility, except for a reduction of N apparent digestibility ($P < 0.01$), but increased efficiency of N utilization ($P = 0.001$) without affecting microbial N production ($P = 0.82$). Total VFA concentration was not ($P = 0.56$) influenced by CP content. Compared with GSS, RSE exhibited a greater neutral detergent fibre digestibility ($P < 0.01$), it did not influence total volatile fatty acids (VFA; $P = 0.10$) but decreased the proportions of acetate and propionate on total VFA ($P < 0.001$) and increased those of butyrate and branched-chain VFA ($P < 0.001$). Microbial efficiency was comparable for GSS and RSE. Results suggest that reduction of dietary CP concentration in DM did not impair in vitro digestibility and microbial growth. The protein mixture containing homemade RSE showed in vitro fermentative properties and microbial growth comparable with those of GSS.

Key words: Rapeseed expellers, RUSITEC, microbial growth, rumen degradability

Guadagnin, M., Tagliapietra, F., Cattani, M., Schiavon, S., Worgan, H. J., Belanche, A., Newbold, C. J. et Bailoni, L. 2013. **Fermentation ruminale et rendement microbien des diètes à haute ou faible concentration en protéines et contenant des graines de soya moulues ou des tourteaux de colza de fabrication artisanale évalués avec RUSITEC**. Can. J. Anim. Sci. **93**: 363–371. Le but de cette expérience était de comparer des diètes contenant deux concentrations de protéines brutes (PB; 147 ou 109 g kg^{-1} de matière sèche, MS) et deux sources de protéines contenant des graines de soya moulues (GSS) ou du tourteau de colza (RSE). La digestibilité, les acides gras volatils (AGV), les concentrations en ammoniac et les flux d'azote des diètes ont été comparés en utilisant des fermenteurs qui simulent le rumen (RUSITEC). Du RSE de fabrication artisanale (PB = 287 g kg^{-1} MS et EE = 199 g kg^{-1} MS) a été produit en utilisant de l'équipement adopté par des petites fermes. La réduction du contenu en PB alimentaires n'a pas affecté la digestibilité, excepté pour une réduction de la digestibilité apparente de N[GC1] ($P < 0{,}01$). Par contre, la réduction du contenu en PB a augmenté l'efficacité de l'utilisation de N ($P = 0{,}001$) sans affecter la production microbienne de N ($P = 0{,}82$). La concentration d'AGV totaux n'a pas été influencée ($P = 0{,}56$) par le contenu en PB. Comparée aux GSS, la digestibilité des NDF[GC2] du RSE a été plus élevée ($P < 0{,}01$). Le RSE n'a pas influencé les acides gras volatils totaux (AGV; $P = 0{,}10$) mais a diminué les proportions d'acétate et de propionate ($P < 0{,}001$) et augmenté celles du butyrate et des AGV à chaîne branchée ($P < 0{,}001$). L'efficacité microbienne était comparable pour GSS et RSE. Les résultats suggèrent que la réduction de la concentration des PB

Abbreviations: **ADF**, acid detergent fibre expressed inclusive of residual ash; **CP**, crude protein; **DM**, dry matter; **DMD**, DM digestibility; **dNDF**, digestible NDF; **EE**, ether extract; **EMS**, efficiency of microbial synthesis; **E-NAN$_{TD}$**, non ammonia nitrogen of total digesta ^{15}**N** enrichment; **E-NH$_3$**, ammonia nitrogen ^{15}**N** enrichment; **ENU**, efficiency of nitrogen utilization; **E-TB**, total bacteria ^{15}**N** enrichment; **MN**, microbial nitrogen; **NAD**, nitrogen apparent digestibility; **NAN**, non-ammonia nitrogen; **NDF**, neutral detergent fibre assayed with a heat stable amylase and expressed inclusive of residual ash; **N-NH$_3$**, ammonia N; **OM**, organic matter; **OMAD**, OM apparent digestibility; **TOMD**, true OM digestibility; **VFA**, volatile fatty acid

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alimentaires n'a pas compromis la digestibilité in vitro et la croissance microbienne. Les propriétés de fermentation in vitro et la croissance microbienne des mélanges de protéines contenant du RSE de fabrication artisanale ont été comparables à celles des GSS.

Mots clés: Tourteau de colza, RUSITEC, croissance microbienne, dégradabilité ruminale

Recent constraints introduced by the Nitrate Directive (European Commission 1991) and the increasing market price of soybean products are forcing farmers to consider the use of low-protein diets (Dal Maso et al. 2009; Schiavon et al. 2010, 2012) and alternative protein sources (McKinnon and Walker 2009). Recent work conducted on double-muscled Piemontese bulls evidenced as a reduction in dietary crude protein (CP) concentration in dry matter (DM) from 147 to 108 g kg^{-1} did not influence DM intake, total volatile fatty acids concentration in the rumen fluid, nutrient digestibility, average daily gain or nitrogen retention (Schiavon et al. 2010, 2012). Based on these results we hypothesized that in vitro a reduction of dietary CP concentration in DM from 147 to 109 g kg^{-1} of DM would exert little effect on rumen fermentation, feed digestibility or microbial growth.

Rapeseed meal, a by-product obtained from the solvent-extraction of oil from rapeseeds, has been successfully used to replace soybean meal in beef cattle diets (Petit and Veira 1994; Koenig and Beauchemin 2005). However, less information is available about rapeseed expellers, obtained as a by-product from the mechanical extraction of oil from seeds (Thacker and Petri 2009; Kaldmäe et al. 2010). Rapeseed expellers show a variable chemical composition, as the mechanical extraction of oil is less standardized compared with solvent-extraction. In this regard, the literature indicates that the fat content of rapeseed expellers can vary from 80 to almost 300 g kg^{-1} of DM (Leming and Lember 2005; Spragg and Mailer 2007; Thacker and Petri 2009). In recent years, an increasing number of farms have also introduced small-sized equipment for mechanical extraction of oil from rapeseed, to produce homemade expellers. In this case seeds are usually pressed at moderate temperatures ($<100^{\circ}C$) using the procedure of ''cold-pressing'', as it uses only the heat generated from seed crushing (McKinnon and Walker 2009). After extraction, the oil is used as fuel for tractors, whereas residual expellers are recycled as protein and fat supplements for animal feeding, to the economic and environmental benefit of local farmers (Baquero et al. 2010; Esteban et al. 2011). The nutritional characteristics of homemade rapeseed expellers are poorly characterized particularly with regard to their influences on rumen fermentation and microbial growth (McKinnon and Walker 2009; Kaldmäe et al. 2010).

Thus, the aim of current experiment was to compare diets differing in dietary CP concentration in DM (high or low) and composed of two different protein sources [ground soybean seeds (GSS) or homemade rapeseed expellers (RSE)] in terms of digestibility, end-products of fermentation, and nitrogen flows, using RUSITEC apparatuses.

MATERIALS AND METHODS

Equipment

All procedures were carried out in accordance with protocols approved by the Aberystwyth University Local Ethical Review Committee. The study was conducted using two rumen simulation apparatuses (RUSITEC) made up of eight fermentation vessels each (Czerkawski and Breckenridge 1977). Each fermentation vessel had a nominal volume of 700 mL. Rumen solid contents for incubation were collected before morning feeding from four dry ruminally fistulated Holstein-Friesian cows fed a standard mixed diet (70% roughages and 30% concentrates) at maintenance level. After the collection, rumen fluid was poured into thermal flasks preheated at $39 \pm 0.1^{\circ}C$ under anaerobic conditions and transferred to the laboratory within 30 min. Artificial saliva (McDougall 1948) was continuously infused by an automatic pump (205U, Watson-Marlow Ltd., Falmouth, Cornwall, UK) in the system (dilution rate: 27.5 mL h^{-1}; 94% outflow rate) and saliva was replaced daily to avoid microbial contamination.

Protein Sources and Experimental Diets

Ground soybean seeds were collected from the experimental farm of the University of Padova (Legnaro, Padova, Italy). Seeds belonging to a variety of winter rapeseed (Excalibur; Dekalb, Monsanto Agricoltura Italia, Milan, Italy) were crushed at moderate temperatures ($60^{\circ}C$) using a small-size apparatus consisting of two single-screw presses equipped with a rotating screw shaft within an horizontal barrel (Mailca Srl, Modena, Italy). The two presses had an input flow rate of 120 kg h^{-1} of seeds, with a production of 40 kg h^{-1} of oil and 80 kg h^{-1} of RSE, and for the production of rapeseed expellers of the current experiment a single pressing step was used.

Four diets, composed of feed ingredients commonly used for beef cattle in Northern Italy, were formulated to contain 147 or 109 g kg^{-1} of CP (on DM basis) by including 140 or only 70 g kg^{-1} DM of GSS or RSE, respectively. Small amounts of soybean meal were also included to achieve the two dietary CP contents (Tables 1 and 2).

Incubation Procedures

The whole incubation lasted 17 d (10 d for adaptation and 7 d for sample collection). The adaptation period

Table 1. Chemical composition of the two protein feed ingredients ($n = 3$, DM basis)

	Ground soybean seed	Rapeseed expeller
Dry matter (g kg^{-1})	888	895
Crude protein (g kg^{-1} DM)	385	287
Starch (g kg^{-1} DM)	42	129
Ether extract (g kg^{-1} DM)	202	199
Neutral detergent fibre (g kg^{-1} DM)	135	291
Acid detergent fibre (g kg^{-1} DM)	62	202
Acid detergent lignin (g kg^{-1} DM)	12	75
Ash (g kg^{-1} DM)	52	66
NDICP[z] (g kg^{-1} DM)	61	63
ADICP[y] (g kg^{-1} DM)	20	24

[z]Neutral detergent insoluble crude protein.
[y]Acid detergent insoluble crude protein.

was used to confirm proper operation of the system and to adapt the microbial populations to the experimental diets (Belanche et al. 2012). On day 1, each fermentation vessel was filled with 300 mL of rumen fluid, 300 mL of artificial saliva, and two nylon bags (110 × 60 mm, pore size 100 μm), one containing rumen solid contents (60 ± 0.5 g) as starter for fermentation activity, and the other one containing the diet (20 ± 0.5 g DM). The diets, ground by a hammer mill (Pullerisette 19, Fritsch GmbH, Laborgeratebau, Germany) through a screen size of 1 mm, were randomly assigned to each fermentation vessel and incubated in four replicates at 39 ± 0.5°C. On day 2, nylon bags containing rumen solid contents were removed and replaced with a nylon bag containing the diet. From day 3 to the end of experiment (day 17), one of the two nylon bags present in the same vessel was alternatively replaced, resulting in 48 h of incubation time for each nylon bag. During the bag replacement process, fermenters were continuously flushed with CO_2 to maintain anaerobic conditions. During the incubation, nylon bags were gently agitated to simulate the rumen movement. On day 11, a dose of 3.15 mg of ^{15}N [95% enriched $(^{15}NH_4)_2SO_4$] was added into each fermentation vessel to label the NH_3-N pool. Then a solution of $(^{15}NH_4)_2SO_4$ was added to the artificial saliva at a rate of 3.70 mg of ^{15}N L^{-1} (2.44 mg of ^{15}N d^{-1}), to label microbial protein with ^{15}N, and to estimate microbial synthesis. During days 11, 12 and 13, nylon bags were collected after 48 h of incubation, washed twice with artificial saliva and then washed in the cold rinse cycle (20 min) of a washing machine. Residuals were analysed for DM, N, and residual neutral detergent fibre (NDF) to evaluate the corresponding digestibility. On the same days, when nylon bags were replaced, pH of vessel fluids was measured. Moreover, an aliquot (4 mL) of vessel fluid was collected from each fermentation vessel and mixed with 1 mL deproteinizing solution (10% metaphosphoric acid and 0.06% crotonic acid, wt vol^{-1}) to determine VFA concentration. Another aliquot (1 mL) of rumen fluid was collected from each vessel and mixed with 0.6 mL of 25% trichloroacetic acid to determine NH_3 concentration. On days 14, 15 and 16, vessel outflows

Table 2. Ingredients and chemical composition of the four experimental diets ($n = 3$, DM basis)

	Dietary CP content (C)			
	High		Low	
Protein source (S)	GSS[z]	RSE[z]	GSS	RSE
Ingredients				
Corn silage (g kg^{-1})	330	316	376	369
Corn meal (g kg^{-1})	290	278	331	324
Dry sugar beet pulp (g kg^{-1})	105	100	119	117
Wheat bran (g kg^{-1})	24	23	28	27
Wheat straw (g kg^{-1})	40	39	46	45
Vitamin-minerals premix[y] (g kg^{-1})	27	26	30	30
Soybean meal (g kg^{-1})	44	78	–	18
Oilseed rape (g kg^{-1})	–	140	–	70
Ground soybean seed (g kg^{-1})	140	–	70	–
Chemical composition				
Dry matter (g kg^{-1})	890	885	904	902
Crude protein (g kg^{-1})	147	147	109	109
Starch (g kg^{-1})	320	320	358	358
Ether extract (g kg^{-1})	57	57	46	46
Neutral detergent fibre (g kg^{-1})	305	321	328	336
Acid detergent fibre (g kg^{-1})	144	153	152	156
Acid detergent lignin (g kg^{-1})	160	265	177	221
Ash (g kg^{-1})	59	53	62	50

[z]GSS, ground soybean seed; RSE, rapeseed expeller.
[y]Contained per kilogram: 400 000 UI of vitamin A; 40 000 UI of vitamin D3; 1000 mg of vitamin E; 80 mg of vitamin B1; 0.40 mg of vitamin B_{12}; 4000 mg of vitamin PP; 2000 mg of choline chloride ruminally protected; 186 g of calcium; 53 g of sodium; 36 g of magnesium; 8 g of phosphorus; 650 mg of manganese; 100 mg of copper; 1500 mg of zinc; 20 mg of iodine; 12 mg of cobalt; 3 mg of selenium.

were collected and saturated with 10 mL d^{-1} of $HgCl_2$ to stop the fermentation process. Residuals from nylon bags incubated for 48 h were collected for 3 d from each vessel and mixed with 250 mL of their correspondent fermentation fluids to account for both liquid and solid digesta fractions (TD) and homogenized using a Stomacher (260 rpm; 2 min). An aliquot of TD (about 500 g) was wetted and adjusted with 1 M-NaOH to pH 10, and dried at 90°C for 16 h to remove NH_3-N (Firkins et al. 1992), lyophilized, and stored at −20°C until non ammonia nitrogen (NAN) and ^{15}N enrichment (E-NAN_{TD}) were determined. A second aliquot of TD (about 500 g) was used to isolate total bacteria (TB), following the procedure of Hristov et al. (2001). Briefly, the aliquot of TD was centrifuged at $500 \times g$ (5 min; 4°C) to separate undigested feed particles, protozoa and attached bacteria. After that, the supernatant was centrifuged at $30\,000 \times g$ and the pellet was isolated. The pellet was analysed for N and ^{15}N enrichment (E-N_{TB}). An aliquot of supernatant (about 200 g) was acidified with 3 mL H_2SO_4, and stored at −20°C until determination of NH_3-N and ^{15}N enrichment (E-NH_3). For the determination of NH_3-N pool, an aliquot (12 mL) of supernatant was diluted with 8 mL of NaOH (10 M) and NH_3-N was evaporated over 7 d at room temperature and absorbed onto 5-mm glass filter disks impregnated with 15 μL of 2.5 M $KHSO_4$ (Brooks et al. 1989). Disks were dried in a desiccator for 24 h before analysing for ^{15}N enrichment.

Analytical Procedures

All feeds were analysed in triplicate for dry matter [DM; # 934.01; Association of Official Analytical Chemists (AOAC) 2003], crude protein (CP; # 976.05; AOAC 2003), ether extract (EE; # 920.29; AOAC 2003), ash (# 942.05; AOAC 2003), starch (# 948.02; AOAC 2003), and NDF (Mertens 2002). The NDF fraction, inclusive of residual ash, was determined with α-amylase and sodium sulphite using the Ankom^{220} Fibre Analyzer (Ankom Technology®, Macedon, NY). Acid detergent fibre (ADF), expressed inclusive of residual ash, and sulphuric acid lignin ($lignin_{(sa)}$) contents were determined sequentially after NDF determination (Van Soest et al. 1991). The VFA concentration was determined using a Varian CP-8400 gas-chromatograph (Agilent Technologies Inc., Palo Alto, CA) equipped with an auto-sampler and a HP-FFAP column (Agilent Technologies Inc., Palo Alto, CA). Ammonia content was determined by a colorimetric method (Weatherburn 1967). Total N and ^{15}N enrichment of NAN_{TD} (E-NAN_{TD}) of TB (E-TB) and of N-NH_3 (E-NH_3) were established using a N analyser connected to a 20–20 mass spectrophotometer (ANCA/SL, PDZ Europe Ltd., Crewe, Cheshire, UK). The DM digestibility (DMD), OM apparent digestibility (OMAD), true OM digestibility (TOMD), NDF digestibility (dNDF), and nitrogen apparent digestibility (NAD) at 48 h of incubation were calculated using standard equations (Goering and Van Soest 1970). The proportion of NAN_{TD} of microbial origin (MN%) was estimated by dividing the ^{15}N enrichment (atom% excess) computed from the proportion of NAN of digesta from each vessel by the enrichment of TB pellets (MN% = E-NAN_{TD}/E-N_{TB}) (Carro and Miller 1999). Daily MN flow was estimated from total NAN multiplied for the proportion attributed to the microbes (MN flow = NAN/MN%, mg d^{-1}). The proportion of microbial N derived from NH_3-N was estimated dividing ^{15}N enrichment of TB by the enrichment of NH_3-N (E-N_{TB}/E-NH_3, %). The efficiency of microbial synthesis (EMS) was estimated as the ratio of daily amount of MN flow and OM apparently digested in the rumen (g MN kg^{-1} OMAD). The daily efficiency of microbial nitrogen utilization (ENU) computed as grams of microbial N/available N × 100 and available N was computed as dietary N – undegraded N [MN/(N intake – NAN + MN)] as described by Bach et al. (2005).

Statistical Analysis

Data, previously tested for normality by using the Shapiro–Wilk statistic (Shapiro and Wilk 1965), were analysed by the PROC MIXED of SAS software (SAS Institute, Inc. 2005) according to the following model:

$$y_{ijklm} = \mu + C_i + S_j + C \times S_{ij} + RUS_k + Vess(RUS)_{kl} + e_{ijklm}$$

where y is the experimental observation, μ is the overall mean, C is the fixed effect of dietary CP concentration in DM ($i = 1, 2$), S is the fixed effect of protein source ($j = 1, 2$), $C \times S$ is the interaction between dietary CP concentration in DM and protein source; RUS is the random effect of RUSITEC apparatus ($k = 1, 2$), $Vess$ is the random effect of vessel within RUSITEC ($l = 1,\ldots,8$), and e is the residual error used as error line to test the effects of C, S, and $C \times S$. Effects were declared significant at $P < 0.05$.

RESULTS

Chemical Composition of Protein Sources

As evidenced in Table 1, compared with GSS, RSE had a lower CP (287 vs. 385 g kg^{-1} DM, for RSE and GSS respectively) a similar EE (199 vs. 202 g kg^{-1} DM, in the same order), a greater NDF concentration in DM (291 vs. 135 g kg^{-1} DM, in the same order), and a greater proportion of $lignin_{(sa)}$ (75 vs. 12 g kg^{-1} DM, in the same order).

Ammonia Nitrogen and VFA Concentrations

The reduction of dietary CP decreased ($P < 0.001$) ammonia N concentration in the rumen fluid (Table 3). In addition, the decrease in the dietary CP did not influence total VFA concentration ($P = 0.56$), but increased the molar percentage of propionate, decreased butyrate, the (acetate + butyrate)/propionate ratio and the branched-chain VFA ($P < 0.001$ for all variables). The protein source did not affect total VFA ($P = 0.10$), but the

Table 3. Effects of dietary CP content (high or low) and protein source on rumen fermentation pattern measured in the rumen simulation technique (RUSITEC) system

	Dietary CP content (C)							
	High		Low			*P*-value		
Protein source (S)	GSS[z]	RSE[z]	GSS	RSE	SEM	C	S	C × S
pH	6.74	6.74	6.76	6.75	0.021	0.201	0.849	0.565
Ammonia N (mmol d^{-1})	4.21	4.30	2.63	2.77	0.203	<0.001	0.417	0.861
Volatile fatty acids (mmol)	30.0	27.9	28.8	28.0	1.19	0.562	0.102	0.434
Acetate (mol %)	42.1	40.2	42.4	39.3	0.30	0.150	<0.001	0.015
Propionate (mol %)	29.5	26.7	32.4	32.0	0.34	<0.001	<0.001	<0.001
Butyrate (mol %)	11.0	13.5	10.4	11.8	0.35	<0.001	<0.001	0.041
Branched[y] (mol %)	17.3	19.6	14.9	16.9	0.25	<0.001	<0.001	0.675
Ratio acetate/propinate	1.43	1.51	1.31	1.23	0.022	<0.001	0.706	<0.001
Ratio (acetate + butyrate)/propionate	1.81	2.01	1.64	1.60	0.035	<0.001	<0.001	<0.001

[z]GSS, ground soybean seed; RSE, rapeseed expeller.
[y]Branched = iso-butyrate + iso-valerate.

replacement of GSS with RSE reduced ($P < 0.001$) the molar percentage of acetate and propionate and increased ($P < 0.001$) butyrate, branched-chain VFA and the (acetate + butyrate)/propionate ratio. The molar proportions of many VFA, but not of total VFA concentration, were affected by the C × S interaction. The replacement of GSS with RSE influenced the (acetate + butyrate)/propionate ratio which was increased in high-CP diets.

Digestibility Values

As reported in Table 4, the reduction of the dietary CP concentration in DM did not affect digestibility of chemical constituents, with the exception of NAD, which was higher ($P < 0.001$) with lower dietary CP concentration in DM.

The replacement of GSS with RSE increased ($P < 0.01$) NAD and dNDF but only tended to increase TOMD ($P = 0.055$). No differences due to the protein source were detected for the values of DMD and OMAD. Similarly, no significant differences were observed for $C \times S$ interaction.

Nitrogen Flows

The reduction of the dietary CP concentration in DM decreased ($P < 0.001$) E-NAN_{TD}, E-TB, and E-NH_3 but did not influence MN flow ($P = 0.82$), as evidenced in Table 5. Conversely, the reduction of the dietary CP concentration in DM increased the MN/NAN ratio ($P = 0.046$) and ENU ($P = 0.001$), but did not influence the proportion of MN originating from ammonia ($P = 0.10$) or EMS calculated as MN per unit of OMAD ($P = 0.71$).

The replacement of GSS with RSE increased E-NAN_{TD} ($P = 0.005$), but did not influence E-TB ($P = 0.45$), E-NH_3 ($P = 0.09$) or MN flow ($P = 0.69$). On the contrary, NAN flow was reduced ($P = 0.005$) by RSE inclusion. Protein source did not influence MN/NAN ratio ($P = 0.146$), ENU ($P = 0.383$) and EMS ($P = 0.681$).

DISCUSSION

Effect of the Dietary CP Concentration in DM

The current experiment was conducted using artificial saliva (McDougall 1948) that does not supply N, so that rumen fluid and diets represented the only N sources for microorganisms, with the exception of negligible amounts of ^{15}N (2.44 mg of ^{15}N d^{-1}) added in the form of ammonium sulphate. As indicated by Cone et al. (2005), these conditions are recommended for a

Table 4. Effects of dietary CP content (high or low) and protein source on digestibility values and gas production in the rumen simulation technique (RUSITEC) system

	Dietary CP content (C)							
	High		Low			*P* value		
Protein source (S)	GSS[z]	RSE[z]	GSS	RSE	SEM	C	S	C × S
Digestibility at 48 h (g kg^{-1})								
Dry matter	589	590	580	576	1.4	0.232	0.882	0.786
Apparent organic matter	508	510	497	492	1.5	0.228	0.903	0.764
True organic matter	799	812	800	807	0.7	0.535	0.055	0.615
Neutral detergent fibre	490	533	509	540	2.1	0.354	0.008	0.643
Nitrogen apparent digestibility	402	466	436	485	1.4	<0.001	<0.001	0.068

[z]GSS, ground soybean seed; RSE, rapeseed expeller.

Table 5. Effects of dietary CP content (high or low) and protein source on ^{15}N enrichment of different N pools

	Dietary CP content (C)							
	High		Low			*P* value		
Protein source (S)	GSS[z]	RSE[z]	GSS	RSE	SEM	C	S	C × S
E-NAN$_{TD}^{y}$	0.21	0.24	0.10	0.15	0.015	<0.001	0.005	0.520
E-TB[x]	0.64	0.52	0.34	0.40	0.061	<0.001	0.449	0.050
E-NH$_3^{w}$	1.80	1.85	1.29	1.50	0.099	<0.001	0.088	0.261
NAN flow[v] (mg d^{-1})	270	222	185	151	22.1	0.086	0.005	0.086
MN flow[u] (mg d^{-1})	97.5	111.2	98.9	100.8	27.19	0.820	0.693	0.765
MN/NAN ratio[t] (%)	38.9	46.0	52.6	73.6	0.02	0.046	0.146	0.450
ENU[s] (%)	91.9	93.9	95.6	96.9	1.34	0.001	0.383	0.579
MN derived from N-NH$_3^{r}$ (%)	33.1	25.4	40.6	31.8	5.51	0.102	0.057	0.881
EMS[q] (g kg^{-1})	15.7	13.5	13.4	13.6	3.80	0.717	0.681	0.745

[z]GSS, ground soybean seed; RSE, rapeseed expeller.
[y]Nonammonia nitrogen of total digesta ^{15}N enrichment (atoms% excess).
[x]Total bacteria ^{15}N enrichment (atoms% excess).
[w]Ammonia nitrogen ^{15}N enrichment (atoms% excess).
[v]Nonammonia N outflow from the vessel.
[u]Microbial N outflow from the vessel.
[t]Computed as microbial N/non ammonia N.
[s]Efficiency of N utilization = g MN g^{-1} rumen-available N × 100.
[r]MN derived from N-NH$_3$ = proportion of MN synthesized using NH$_3$ as a source of N (E-TB/ E-NH$_3$).
[q]Efficiency of microbial synthesis (MN/OMAD).

reliable evaluation of the effects of different dietary CP levels and of different protein sources in rumen fermentation studies. The values obtained for MN flow and EMS averaged 102 mg d^{-1} and 14.1 g kg^{-1}, respectively, without influences due to the dietary CP or the protein sources. These values were similar to those found in other studies conducted with RUSITEC (Lee et al. 2003; Martinez et al. 2010).

In the low-CP compared with the high-CP diet the proportion of starch was increased from 320 to 358 g kg^{-1} DM and the proportion of EE was slightly decreased from 57 to 46 g kg^{-1} DM, whereas other chemical constituents, including NDF, were subjected to small variations. The lower contents of CP and EE, and the greater content of starch for the low-CP diets were reflected in lower ammonia flow and in different VFA molar proportions, with an increase of propionate and a decrease of branched-chain VFA with respect to the high-CP diet. A lower proportion of branched-chain VFA in the low-CP diet was expected, as these substances mostly originated from the deamination of amino acids (McCollum et al. 1987).

Irrespective of the protein source used, the digestibility of the various chemical constituents was not influenced by the reduction of the dietary CP concentration in DM, with the exception that the apparent digestibility of N was slightly increased. The total NAN flow, composed of the sum of dietary and microbial NAN, averaged 246 and 168 mg d^{-1} for the high-CP and the low-CP diets, respectively. This difference was due to differences in the amount of dietary NAN, as the flow of microbial N of the two diets was similar (102 mg d^{-1}). Thus, from the results of the present experiment, it is evident that the flow of microbial protein mass was not influenced by the strong reduction of the dietary CP, and efficiency of nitrogen utilization was increased. This result suggests that at least some categories of microbes increased their efficiency of using the rumen available N when they were kept under a shortage of available N. This finding was also confirmed by the higher apparent digestibility of N observed for the low-CP diets compared with the high-CP diets. It is often assumed that rumen synthesis of microbial N is primarily influenced by protein degradability of feeds (Bach et al. 2005). However, Belanche et al. (2012) found that some non-cellulolytic bacteria have a relatively low constant of ammonia saturation, which allows them to scavenge and to incorporate ammonia even when ammonia is present at low concentrations, and they indicated that a moderate N underfeeding would not affect the rumen concentration of non-cellulolytic microbes or the total tract digestibility. Others described a decrease in fibre and OM digestibility when ruminants have a limited N supply (Doreau et al. 1990), although the reasons underlying this pattern are still unclear. Belanche et al. (2012) suggested that the negative effect of N shortage would mainly reflect the sensitivity of fibrolytic microorganisms and of some minority microbial groups. Thus, a shortage of dietary N would have proportionally greater negative effects when high-forage rather than high-concentrate diets are used, and it might be speculated that a N shortage would exert minor effects on beef cattle fed concentrate diets compared with dairy cows fed diets with high proportions of forages. The results of Belanche et al. (2012) and those obtained in the current experiment are in agreement with findings

with beef cattle, where a dietary CP reduction from 145 to 108 g kg^{-1} DM did not influence growth performance (Schiavon et al. 2010), apparent digestibility, nutrient retention (Schiavon et al. 2012) or meat quality (Schiavon et al. 2011).

Effects of Protein Source

The chemical and physical characteristics of rapeseed expellers produced by the industry or by farms using small apparatuses are strongly influenced by processing conditions such as pressure, temperature and exposure times (Weigal 1991; Mustafa et al. 2000; Glencross et al. 2004a, b). In the current experiment, the homemade RSE evidenced a resulting EE concentration in DM around 200 g kg^{-1} of DM, which was higher than the average of 158 ± 43 g EE kg^{-1} found by Kaldmäe et al. (2010), but similar to that of ground soybean seed used in this experiment.

The results of the current experiment suggest that the replacement of GSS with RSE would exert little influence on the digestibility of most nutrients or total VFA concentration, even if alterations in rumen fermentation occurred due to higher dNDF (+7%) and NAD (+14%), and by changes in the relative proportions of various VFA. It is also indicated that the replacement of GSS with RSE would have little consequence on microbial N flow (despite the observed reduction in the NAN flow) or on the various indexes of N efficiency. Differences observed in the various rumen parameters suggest that rumen microbes used the N supplied by RSE and GSS differently. The increase in the molar concentrations of butyrate and branched-chain VFA achieved when GSS was replaced by RSE is in agreement with the observed increase in dietary protein digestibility. When the microbes were fed RSE the proportion of MN originating from ammonia tended to be lower and the proportion of MN originating from NAN tended to be greater than the corresponding values observed for GSS. Some authors (Salter et al. 1979; Wallace 1997) observed that the proportion of microbial N derived from ammonia depends on the availability of N sources. In particular, they estimated that the minimum contribution to microbial N from ammonia was 0.26 when high amounts of peptides and AA were available, with a potential maximum of 1.0 when ammonia was the sole N source. Accordingly, Nolan and Leng (1972; quoted by Carro and Miller 1999) reported that ammonia was the most common N source for microbial protein synthesis when NAN sources were not readily available. This is in agreement with the results of the present experiment which suggest that when GSS is replaced by RSE, microbes would increase their use of NAN and reduce that of NH_3, at least under in vitro conditions.

CONCLUSION AND IMPLICATIONS

The results of the current experiment indicate that in beef cattle concentrate diets, the reduction of the dietary CP concentration in DM from 147 to 109 g kg^{-1} did not exert negative influences on in vitro nutrient digestibility or microbial growth. This supports the hypothesis that such reduction has little effect on rumen fermentation, feed digestibility and microbial growth. Such findings might be explained by the ability of certain non-cellulolytic bacteria to scavenge and incorporate ammonia even when ammonia is available at low concentrations, and further studies are required to clarify the causes of these results. In growing cattle fed concentrate diets, some N underfeeding would be of benefit for those farms that must reduce N excretion for the environmental constraints introduced by law in areas where N pollution is of concern.

It is also suggested that rumen fermentation, feed digestibility, microbial protein synthesis and overall value of beef cattle concentrate diets would not be influenced by the replacement of ground soybean seed with rapeseed expellers. Considering that the replacement, at least in part, of soybean products with homemade rapeseed expellers is considered an important task to improve the profitability of beef farms, conformation of these results with in vivo experiments is desirable.

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