Research Article

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In situ ruminal disappearance of crude protein and phytate from differently processed rapeseed meals in dairy cows

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Abstract

BACKGROUND: The influence of different processing conditions of rapeseed meal on ruminal degradation of crude protein and phytate in dairy cows was investigated. Following oil extraction from the rapeseed, five residence times in the desolventizer/toaster were chosen to remove the solvent from the meal. Rapeseed cake and rapeseed meals were incubated *in situ* in the rumen of three fistulated dairy cows to determine ruminal degradation parameters.

RESULTS: With increasing residence time in the desolventizer/toaster the ruminal degradation of crude protein decreased significantly for every treatment step. Ruminal phytate degradation and crude protein degradation were affected almost identically.

CONCLUSION: The processing conditions of rapeseed meal have a major impact on the ruminal degradation of crude protein and phytate, indicating a potential conflict of interest regarding the production process. Large amounts of undegradable rumen protein are often intended for high-yielding dairy cows whereas a high level of ruminal degradation is preferred for phytate to increase absorption of phosphorus in the small intestine.

Keywords: *in situ*; rumen; crude protein; phytate; rapeseed meal; processing conditions

INTRODUCTION

Rapeseed meal (RSM) is a widely used protein feed for dairy cows and its protein value can compete with that of soybean protein.[1] Ruminal *in situ* studies have shown that the degradation rate and the effective degradation of crude protein (CP) is higher and the percentage of rumen undegradable protein (RUP) is lower in soybean meal (SBM) than in RSM.[2] *In vivo* studies have also confirmed that the amount of RUP provided by RSM is at least equal to, if not greater than, that of SBM.[1] The extent of ruminal CP degradation and, thus, the RUP content is mainly influenced by processing conditions, however. Physical and chemical treatments of protein feeds such as heat and formaldehyde are established means to increase RUP concentrations and their respective effects on RSM have been reported.[3-5] Conditions during the manufacturing of RSM seem to determine the concentration of RUP, as studies with RSM from different oilseed plants have suggested.[6,7] When ten RSM samples from ten different oilseed plants were examined in an *in situ* study, the effective ruminal degradation of CP (EDCP), calculated for a ruminal outflow rate of 8% h^{-1}, ranged from 440 to 630 g kg^{-1} CP.[6] Substantial RUP differences in RSM were observed consistently when RSM samples from 12 processing plants were collected and analyzed *in vitro* repeatedly for 4 years.[7] In that study, the RUP content ranged from 410 to 490 g kg^{-1} CP for the examined RSM. Over the years, no differences in RUP were observed for the RSM from the same plant. Except for one sample in a study by Broderick *et al*,[7] where the oil was removed using expeller extraction, the RSM was extracted using solvents in both studies.[6,7] Thus, even when using the same extraction procedure, differing conditions during extraction can lead to remarkable differences in the RUP in RSM. The main causes of these differences are probably differences in the level of heating during desolventizing and toasting.[8,9]

If processing conditions affect EDCP of RSM, it can be assumed that the ruminal degradation of other components associated with protein is also affected. In a previous study we found a strong relationship between the ruminal CP and phytate disappearance for oilseed meals.[10] Phytate (any salt of phytic acid; *myo*-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate); $InsP_6$) represents the main storage form of phosphorus (P) in plant seeds and processing by-products. A strong association between $InsP_6$ and protein occurs, especially in rapeseed, as $InsP_6$ is located inside the protein

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storage vacuole[11,12] and is capable of binding rapeseed proteins.[8] Even after flaking, pressing, solvent extraction, and desolventizing/toasting of the rapeseed, $InsP_6$ in RSM remains associated with the denatured protein mass.[12] *In situ* studies examining RSM that has undergone further heat treatment not only showed reduced EDCP but also reduced ruminal $InsP_6$ disappearance.[5,10] For $InsP_6$, however, a high effective ruminal degradation ($EDInsP_6$) is preferable. The P bound in $InsP_6$ ($InsP_6$-P) is available for intestinal absorption only after hydrolytic cleavage catalyzed by $InsP_6$-hydrolysing enzymes.[13] The substantial phytase activity of rumen microorganisms[14] provides cleaved P for absorption in the small intestine, which is the predominant absorption site of ruminants.[15] Post-ruminal $InsP_6$ hydrolysis has been observed mainly in the large intestine[16,17] rendering the P unavailable for absorption. Thus, an increased flow of $InsP_6$ into the large intestine can lead to more fecal excretion of the released P[17] contributing to increased P entry into the environment.

The first objective of the present study was to evaluate the effect of changing the desolventizing/toasting conditions during the manufacturing process of RSM on protein fractions, EDCP and $EDInsP_6$. We also analyzed the concentrations of lower inositol phosphates (InsPs; isomers of $InsP_5$, $InsP_4$, $InsP_3$) to assess whether the pattern of lower InsPs in the RSM produced changes through different desolventizing/toasting conditions leading to an incomplete ruminal hydrolysis of InsPs. We hypothesized that different RSM treatments cause ruminal CP and $InsP_6$ degradation to be affected in a similar manner.

MATERIALS AND METHODS

Examined rapeseed meals and processing procedures

Five differently processed RSM and one rapeseed cake (RSC), which was used to produce four of the RSM samples, were examined. The processing conditions were described in detail in an earlier publication.[18] In brief, non-genetically modified winter-type rapeseed of the Lorenz variety (*Brassica napus* L., seed grade quality) was used in a pilot plant under standardized and defined conditions. The glucosinolate concentration of the rapeseed was 17 μmol g^{-1} DM. After flaking, cooking, and pressing the rapeseed, the resulting RSC was pelleted at very low pressure to homogenize the RSC fragments. The residual oil was then removed with hexane as solvent. The subsequent desolventization in the desolventizer/toaster (DT) differed for the rapeseed meals that were examined. Four RSM samples were produced under wet toasting conditions (indirect heat, 850 kPA, direct unsaturated steam (15 kg h^{-1})) using marc from the examined RSC. The residence time in the DT was 48 (RSM48), 64 (RSM64), 76 (RSM76), and 93 (RSM93) min at an average toasting temperature of 124 °C. Furthermore, a fifth RSM with two additional heating steps in the DT (RSMover) was produced. For that purpose, another batch of rapeseed cake was produced from the same seed and again four RSM were prepared under similar processing conditions to those described above. The four RSM from the second production batch were pooled to one RSM sample, which then was exposed to direct saturated steam (30 kg h^{-1}) and a maximum temperature of 100 °C for 30 min. Afterwards, dry toasting conditions were applied for 30 min at an average temperature of 107 °C.

Animals, housing, and diet

Three ruminally fistulated, mid-lactating Jersey cows were used for the *in situ* incubations. The animals were housed in a freestall barn with cubicles covered with rubber mats and chopped straw and milked twice daily (average milk yield 22 kg day^{-1}). The cows were fed a diet containing 240 g maize silage, 240 g grass silage, 160 g hay, 30 g barley straw, 200 g concentrate mixture consisting of barley, field beans, rapeseed cake, maize, peas, 100 g rapeseed meal, and 30 g additives (vitamins, limestone, salt, P-free mineral feed) kg^{-1} DM. The ration contained 6.2 MJ net energy for lactation and 135 g CP kg^{-1} DM and was provided as a TMR and prepared freshly every morning. The animals had free access to water and TMR and the average DMI of the cows was 17 kg d^{-1}. Housing, diets, and incubation procedure were in accordance with the German Welfare Regulations and approved by the Regierungspräsidium Stuttgart, Germany (approval code V319/14 TE).

Incubation procedure and sample preparation

The incubation procedure was based on the recommendations for a standardized method for measuring ruminal protein degradation of concentrate feeds.[19] Grinding of the six feed samples was not necessary as the particle size was below the recommended sieve size of 2 mm. Due to the small particle size of the samples, polyester bags with a pore size of 30 μm were used. The bags (size: 11 × 22 cm) were sewn manually from 30 μm polyester screen cloth (DIN 4197, Fa. Linker Industrie-Technik, Kassel, Germany). Feed sample (8 g) was weighed into the polyester bags and two bags of each feed sample were attached to a cylindrical weight of approximately 700 g. The samples were incubated in the rumen of each cow for 2, 4, 8, 16, 24, 48, and 72 h. Before inserting them into the ventral sac of the rumen, the bags were soaked in warm water (approximately 39 °C) for 5 min. At the end of the incubation times, bags were removed from the rumen and immediately placed in ice water to minimize further microbial fermentation. The bags were then rinsed with cold tap water to remove adhering particles and afterwards washed in a washing machine for 20 min (2 cycles of rinsing without spinning, each cycle including one water exchange) and dried at 60 °C for 24 h. To determine the initial 0 h time point ($t = 0$), three bags of each sample were rinsed, washed, and dried as described before without ruminal incubation.

Dried bag residues were pooled for each cow and incubation time, providing three replicates for each feed sample and time point. Samples were pulverized in a vibrating disk mill (Pulverisette, Fritsch GmbH, Idar-Oberstein, Germany) and stored at 4 °C until analysis.

Chemical analyses

Dry matter (method 3.1) and CP (method 4.1.1) concentrations of the feed samples and bag residues were analyzed according to the *Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch)* (official methods in Germany).[20] Analysis of inositol phosphates (InsPs), including $InsP_6$ and isomers of lower inositol phosphates (*myo*-inositol pentakisphosphate ($InsP_5$), *myo*-inositol tetrakisphosphate ($InsP_4$), *myo*-inositol trisphosphate ($InsP_3$)) was performed as described by Zeller *et al.*[13] with slight modifications regarding sample size and extracting agent used for extraction as described by Haese *et al.*.[21] The isomers were measured by high-performance ion chromatography (ICS-3000 system, Dionex, Idstein, Germany). It is not possible to separate the enantiomers using this methodology. The results therefore do not distinguish between the D- and L-forms.

Chemical protein fractions in the feed samples (fractions A, B1, B2, B3, C) were calculated according to the Cornell Net

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Carbohydrate and Protein System (CNCPS).[22] Non-protein nitrogen (NPN) and buffer-soluble nitrogen were determined with trichloracetic acid (Merck KGaA, Darmstadt, Germany) as the precipitating agent.[23] To determine neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN), the CP concentration of neutral detergent fibre (aNDF, method 6.5.1, without incineration[20]) and acid detergent fibre (ADF, method 6.5.2, without incineration[20]) was analysed, respectively.[20] According to the CNCPS, fractions A and C are defined as NPN and ADIN, respectively. Fraction B1 represents the rapidly degradable protein and is calculated as the difference between NPN and buffer-soluble protein. Fraction B3 is defined as slowly degradable true protein and was calculated as the difference between ADIN and NDIN. Fraction B2 represents the true protein with intermediate degradation time and was calculated as 1000 – A - B1 - B3 - C.[22]

Calculations and statistical analysis

To obtain the degradation parameters *b* (g kg^{-1}; potentially degradable), *a* + *b* (g kg^{-1}; maximum degradation/plateau), and *c* (% h^{-1}; degradation rate of *b*), ruminal degradation values (Deg, g kg^{-1}) for CP and $InsP_6$ after *t* hours were fitted to exponential equations, including or excluding lag time (L, *lag*). The better fitting model, according to the Akaike information criterion, was then used for further calculations, which were:

$$\text{Deg} = a + b \times (1 - e^{-ct}) \quad (1)$$

as described by Orskov and McDonald[24] for CP, and

$$\text{Deg} = a + b \times \left(1 - e^{-c(t-L)}\right) \text{ for } t > L \quad (2)$$

as described by McDonald[25] for $InsP_6$.

For *a* (g kg^{-1} CP or $InsP_6$, washout fraction defined as instantly disappearing), the values for $t = 0$, determined as described above, were set.

As described by Orskov and McDonald,[24] the degradation values of CP were used to calculate EDCP at ruminal outflow rates of $k = 5$ or 8% h^{-1} (ED_5CP/ED_8CP) with

$$\text{EDCP} = a + [(b \times c)/(c + k)] \quad (3)$$

The $EDInsP_6$ was also calculated at ruminal outflow rates of $k = 5$ or 8% h^{-1} (ED_5InsP_6/ED_8InsP_6) with

$$\text{EDInsP}_6 = a + [(b \times c)/(c + k)]\, e^{-kL} \quad (4)$$

as suggested by Wulf and Südekum.[26]

Degradation parameters *lag*, *b*, *a* + *b*, and *c* as well as EDCP and $EDInsP_6$ were calculated individually for each cow and statistically analyzed using the cow as an experimental unit. In a one-factorial approach with the SAS MIXED procedure (SAS System for Windows, Version 9.4, SAS Institute, Cary, NC, USA), the following model was used:

$$Y_{ij} = \mu + FS_i + A_j + e_{ij} \quad (5)$$

with Y_{ij} as a responsive mean, μ as overall mean, FS_i as fixed effect of feed sample (i = RSC, RSM48, RSM64, RSM78, RSM93, RSMover), A_j as random effect of animal (j = 1, 2, 3), and e_{ij} as residual error. $P \leq 0.05$ was considered statistically significant. Following a significant *F*-value, individual significant differences between means were determined using *t*-tests. Data are presented as least-squares means (LS means) and pooled standard error of the means (pooled SEM).

Correlations between protein fractions and degradation parameters were tested using the PROC CORR procedure of SAS where $P \leq 0.05$ was considered statistically significant.

RESULTS

The CP concentrations in the RSM that was produced were higher in comparison with RSC (367 to 374 versus 295 g kg^{-1} DM, Table 1) and the protein fractions also differed between RSC and the RSM produced thereof (Fig. 1). In comparison with RSC, the desolventizing/toasting process of RSM generally decreased protein fractions with fast and intermediate degradation rate (B1 and B2) and increased slowly degradable and unavailable protein fractions (B3 and C). Among the RSM variants, fractions B1

Table 1. Concentrations of crude protein (CP, g kg^{-1} DM) and inositol phosphates ($InsP_6$, ∑ of $InsP_5$-, $InsP_4$-, $InsP_3$- isomers, and concentrations of different $InsP_5$ isomers, µmol g^{-1} DM) in rapeseed cake (RSC) and five rapeseed meals (RSM) with different residence times in the desolventizer/toaster during the production process

	RSC	RSM48[a]	RSM64[a]	RSM76[a]	RSM93[a]	RSMover[a]
CP	295	371	367	372	372	374
$InsP_6$	32.0	38.2	37.6	35.5	35.1	34.4
$\sum InsP_5$	1.3	8.0	8.8	9.0	10.7	11.9
$\sum InsP_4$	ND[b]	0.7	0.9	1.3	1.9	1.9
$\sum InsP_3$	ND[b]	ND[b]	0.2	0.2	0.4	0.2
$\sum InsP_{3\text{-}6}$	33.3	46.9	47.5	46.0	48.1	48.4
$InsP_5$ isomers						
Ins(1,2,4,5,6)P_5	ND[b]	3.6	3.9	3.9	4.6	5.2
Ins(1,2,3,4,5)P_5	0.9	2.6	2.9	3.0	3.6	3.9
Ins(1,2,3,4,6)P_5	0.4	1.4	1.6	1.6	2.0	2.2
Ins(1,3,4,5,6)P_5	ND[b]	0.4	0.4	0.5	0.5	0.6

[a] RSM48, 48 min residence time; RSM64, 64 min residence time; RSM76, 76 min residence time; RSM93, 93 min residence time; RSMover, blend of RSM manufactured in a similar way to RSM48, RSM64, RSM76, RSM93, and further heat treated for 60 min.
[b] ND, not detected.

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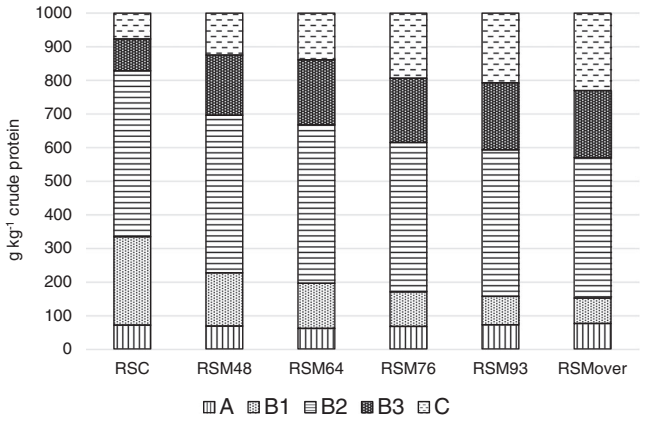

Figure 1. Chemical protein fractions A, B1, B2, B3, C (g kg^{-1} of crude protein) in rapeseed cake (RSC) and five rapeseed meals (RSM) with different residence times in the desolventizer/toaster during the production process of the meals. Residence times were 48 min (RSM48), 64 min (RSM64), 76 min (RSM76), and 93 min (RSM93); for RSMover, a blend of RSM manufactured in a similar way to RSM48, RSM64, RSM76, and RSM93 was prepared and further heat treated for 60 min.

and B2 decreased with increasing residence time in the DT (B1: 166 to 88 g kg^{-1} CP; B2: 470 to 420 g kg^{-1} CP for RSM48 and RSMover, respectively) while fraction C increased markedly (120 to 230 g kg^{-1} CP in RSM48 and RSMover, respectively). Fractions A and B3, however, were hardly influenced by heat exposure (A: 60 to 80 g kg^{-1} CP; B3: 180 to 200 g kg^{-1} CP).

The washout fraction *a* of CP was lower for the examined RSM than for RSC and decreased with increasing residence time in the DT (RSC: 383 g kg^{-1} CP; RSM48: 256 g kg^{-1} CP; RSMover: 100 g kg^{-1} CP, Table 2). The opposite effect was observed for the potentially degradable fraction *b* of CP, which increased with intensified processing conditions (RSC: 565 g kg^{-1} CP; RSM48: 657 g kg^{-1} CP; RSMover: 773 g kg^{-1} CP, Table 2). The degradation rate *c* declined significantly ($P < 0.001$) from 18% h^{-1} for RSC to 14% h^{-1} for RSM48 and 6.7% h^{-1} for RSMover. As a result, the EDCP was significantly higher ($P < 0.001$) in RSC (ED_8CP: 765 g kg^{-1} CP) compared to the RSM and declined further with every step of heat treatment (ED_8CP: 670 g kg^{-1} CP RSM48; 455 g kg^{-1} CP RSMover; Table 2).

The concentration of InsPs ($\sum InsP_{3\text{-}6}$) in the produced RSM were higher than RSC (46.0 to 48.4 versus 33.3 μmol g^{-1} DM, Table 1). Residence time in the DT changed the pattern of InsPs in the RSM leading to lower concentrations of $InsP_6$ with increasing heat exposure (RSM48: 38.2 μmol g^{-1} DM; RSMover: 34.4 μmol g^{-1} DM). The concentrations of total $InsP_5$ ($\sum InsP_5$) and $InsP_4$ isomers ($\sum InsP_4$) increased with longer residence time in the DT ($\sum InsP_5$: 8.0 (RSM48) to 11.9 μmol g^{-1} DM (RSMover); $\sum InsP_4$: 0.7 (RSM48) to 1.9 μmol g^{-1} DM (RSMover), Table 1). Minor concentrations of $InsP_3$ isomers (0.2 to 0.4 μmol g^{-1} DM) were detected only in RSM with a residence time in the DT of >60 min. In RSC, the $InsP_5$ isomers detected were Ins(1,2,3,4,5)P_5 and Ins(1,2,3,4,6)P_5 whereas Ins(1,2,4,5,6)P_5 and Ins(1,3,4,5,6)P_5 were also detected in RSM (Table 1).

A lag phase of 2.7 to 4.7 h was observed for $InsP_6$ degradation in all the examined rapeseed feeds but the differences were not significant ($P = 0.128$) between treatments (Table 3). The treatments exerted similar effects on $InsP_6$ degradation as on CP degradation. The washout fraction *a* of $InsP_6$ was markedly lower for RSMover (33 g kg^{-1} $InsP_6$) compared with RSC and the other RSM (210 to 283 g kg^{-1} $InsP_6$, Table 3). In comparison with RSC, fraction *b* was lower for RSM48, RSM64, and RSM76 (675 to 693 versus 761 g kg^{-1} $InsP_6$; $P < 0.001$). For RSM93, no difference occurred for fraction *b* (744 g kg^{-1} $InsP_6$) in comparison with RSC and fraction *b* was significantly higher for RSMover (917 g kg^{-1} $InsP_6$). The degradation rate *c* as well as EDInsP$_6$ was lower for the examined RSM compared with RSC and declined significantly ($P < 0.05$) with almost every step of heat treatment (c: 45 to 7.6% h^{-1}; ED_8InsP_6: 722 to 352 g kg^{-1} $InsP_6$ for RSC and RSMover, respectively, Table 3). At any incubation time, $InsP_6$ was the most abundant inositol phosphate in the bag residues of all incubated rapeseed feeds (Fig. 2), making up at least 57% of total InsPs. No increase in the $\sum InsP_5$ isomer concentration was observed during the incubation process except for RSMover, where values increased slightly from 18.2 to 21.5 μmol g^{-1} DM between 2 and 8 h of incubation. Only very low concentrations of $InsP_4$ and $InsP_3$ isomers were detected in the bag residues, ranging from 0 to 3.6 μmol g^{-1} DM for $\sum InsP_4$ and from 0 to 0.6 μmol g^{-1} DM for $\sum InsP_3$.

Linear regressions were performed to evaluate correlations between degradation parameters of CP and $InsP_6$. Besides the examined RSC and RSM in the present study, three further RSM from earlier *in situ* studies in our laboratory were included in the regression analysis. Two of the additional RSM were from the same batch with or without additional heat treatment,[10] the third RSM was a commercial, formaldehyde treated product (1.2 kg formaldehyde kg^{-1}, Byoprofin-R®, Fa. Wulfa, Dinklage-Wulfenau,

Table 2. *In situ* degradation parameters (*a*, *b*, *a* + *b* in g kg^{-1} CP, *c* in % h^{-1}) of crude protein (CP, n = 3) and effective ruminal degradation of CP, calculated for ruminal outflow rates of 5% h^{-1} (ED_5CP, g kg^{-1} CP) and 8% h^{-1} (ED_8CP, g kg^{-1} CP), of rapeseed cake (RSC) and five rapeseed meals (RSM) with different residence times in the desolventizer/toaster during the production process (LS means and pooled SEM)

	RSC	RSM48†	RSM64†	RSM76†	RSM93†	RSMover†	pooledSEM
a‡	383	256	204	183	156	100	
b‡	565^{e}	657^{d}	709^{b}	680^{c}	724^{b}	773^{a}	8.554
a + *b*‡	941^{a}	909^{b}	905^{b}	860^{c}	875^{c}	876^{c}	8.554
c‡	18^{a}	14^{b}	13^{b}	11^{c}	9.2^{c}	6.7^{d}	0.799
ED_5CP	816^{a}	736^{b}	711^{c}	642^{d}	619^{e}	546^{f}	6.625
ED_8CP	765^{a}	670^{b}	638^{c}	568^{d}	538^{e}	455^{f}	7.003

Different superscript letters within a row indicate significantly different LS means ($P \leq 0.05$).
† RSM48, 48 min residence time; RSM64, 64 min residence time; RSM76, 76 min residence time; RSM93, 93 min residence time; RSMover, blend of RSM manufactured in a similar way to RSM48, RSM64, RSM76, RSM93, further heat treated for 60 min.
‡ *a* = washout fraction, *b* = potentially degradable fraction, *a* + *b* = plateau, *c* = degradation rate of *b*.

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Table 3. *In situ* degradation parameters (*lag* in h, *a*, *b*, *a* + *b* in g kg^{-1} $InsP_6$, *c* in % h^{-1}) of phytate ($InsP_6$, *n* = 3) and effective ruminal degradation of $InsP_6$, calculated for ruminal outflow rates of 5% h^{-1} (ED_5InsP_6, g kg^{-1} $InsP_6$) and 8% h^{-1} (ED_8InsP_6, g kg^{-1} $InsP_6$) of rapeseed cake (RSC) and five rapeseed meals (RSM) with different residence times in the desolventizer/toaster during the production process (LS means and pooled SEM)

	RSC	RSM48[†]	RSM64[†]	RSM76[†]	RSM93[†]	RSMover[†]	pooledSEM
lag[‡]	2.7[a]	4.4[a]	4.0[a]	4.4[a]	4.7[a]	4.2[a]	0.749
a[‡]	237	274	283	277	210	33	
b[‡]	761[b]	693[c]	685[c]	675[c]	744[b]	917[a]	7.844
a + *b*[‡]	998[a]	967[b]	968[b]	952[b]	954[b]	950[b]	7.844
c[‡]	45[a]	27[ab]	20[bc]	12[c]	11[c]	7.6[c]	7.916
ED_5InsP_6	806[a]	742[b]	729[b]	660[c]	617[d]	481[e]	12.73
ED_8InsP_6	722[a]	650[b]	635[b]	563[c]	509[d]	352[e]	15.07

Different superscript letters within a row indicate significantly different LS means ($P \leq 0.05$).
[†] RSM48, 48 min residence time; RSM64, 64 min residence time; RSM76, 76 min residence time; RSM93, 93 min residence time; RSMover, blend of RSM manufactured similarly as RSM48, RSM64, RSM76, RSM93, further heat treated for 60 min.
[‡] *lag*, lag phase before onset of degradation, *a* = washout fraction, *b* = potentially degradable fraction, *a* + *b* = plateau, *c* = degradation rate of *b*.

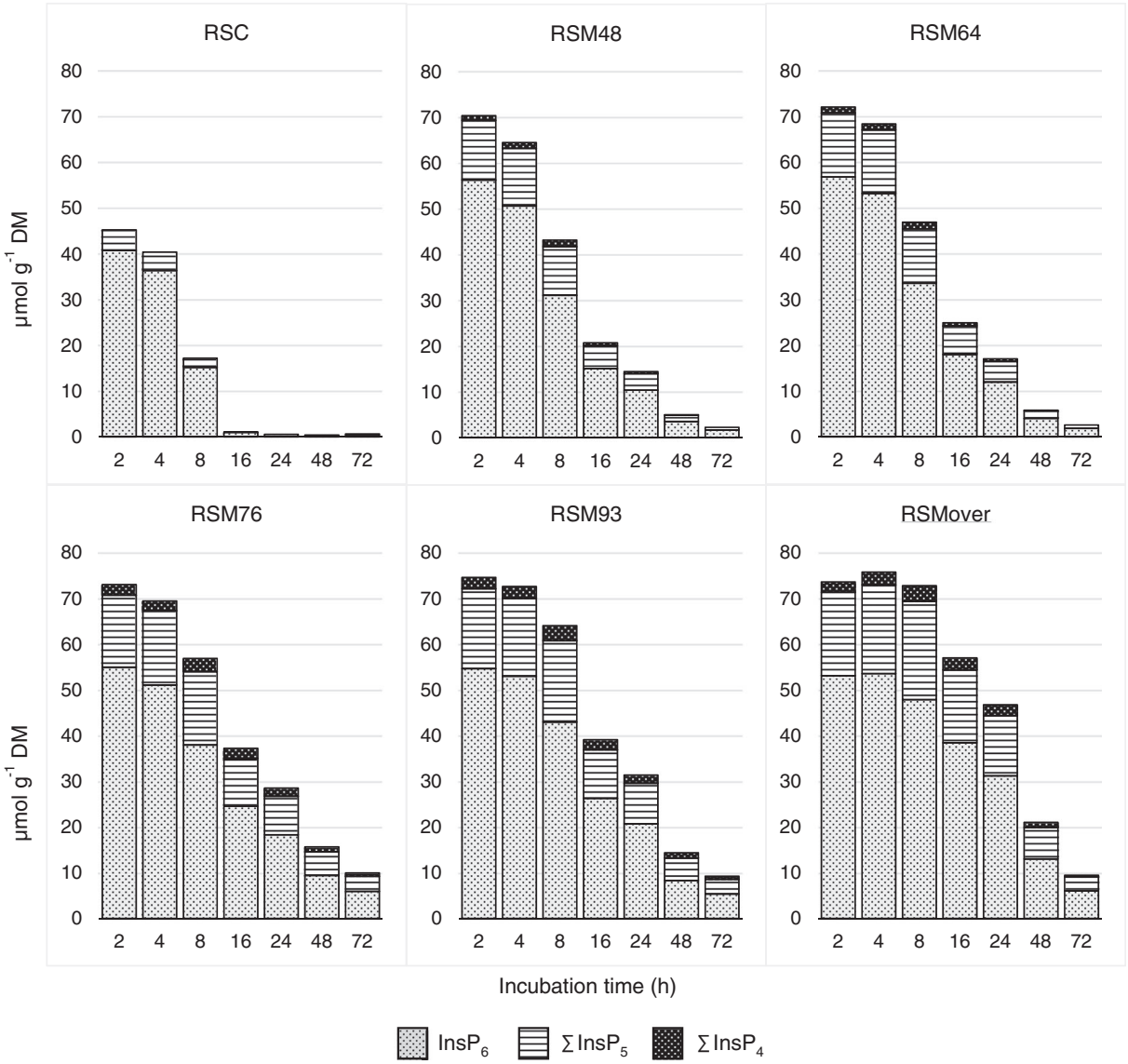

Figure 2. Concentrations of inositol phosphates ($InsP_6$, ∑ of $InsP_5$- and $InsP_4$-isomers; µmol g^{-1} DM) of rapeseed cake (RSC) and five different rapeseed meals (RSM) in the bag residues after ruminal incubation for 2, 4, 8, 16, 24, 48, and 72 h. During the production process of the RSM, different residence times in the desolventizer/toaster were applied: 48 min (RSM48), 64 min (RSM64), 76 min (RSM76), and 93 min (RSM93). For RSMover, a blend of RSM manufactured in a similar way to RSM48, RSM64, RSM76, and RSM93 was prepared and further heat treated for 60 min.

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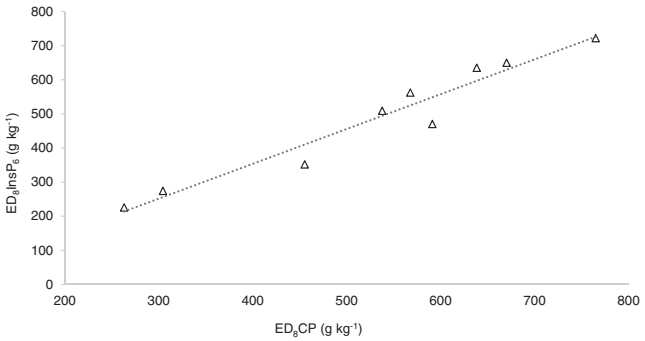

Figure 3. Linear regression for the effective ruminal degradation of crude protein (ED_8CP) and phytate (ED_8InsP_6) from nine different rapeseed feeds, calculated for a rumen outflow rate of 8% h^{-1} ($y = 1.02x - 54.5$; $R^2 = 0.94$; RMSE = 44.0; SE = 0.09).

Table 4. Correlations between protein fractions (A, B1, B2, B3, C) and the degradation rate (*c*) of the potentially degradable fraction of phytate ($InsP_6$) or the effective ruminal degradation of $InsP_6$, calculated for ruminal outflow rates of 5% h^{-1} (ED_5InsP_6) and 8% h^{-1} (ED_8InsP_6), of rapeseed cake (RSC) and five rapeseed meals (RSM) with different residence times in the desolventizer/toaster during the production process ($n = 6$)

	c	ED_5InsP_6	ED_8InsP_6
A	n.s.[a]	n.s.[a]	n.s.[a]
B1	0.994***	0.837*	0.831*
B2	0.927**	0.969**	0.965**
B3	−0.925**	n.s.[a]	n.s.[a]
C	−0.963**	−0.938**	−0.932**

*$P \leq 0.05$; ** $P \leq 0.01$; ***$P \leq 0.001$.
[a] n.s., not significant.

Germany, unpublished). High coefficients of determination were calculated for the linear regression of ED_8CP/ED_8InsP_6 ($y = 1.02x - 54.5$; $R^2 = 0.94$; RMSE = 44.0; SE = 0.09; Fig. 3) and ED_5CP/ED_5InsP_6 ($y = 1.01x - 23.8$; $R^2 = 0.95$; RMSE = 39.4; SE = 0.09; data not shown).

Strong and highly significant correlations were observed between the degradation rate *c* of $InsP_6$ and the protein fractions B1, B2, B3, and C (Table 4). Between $EDInsP_6$ and fractions B2 and C, correlations were also strong and highly significant at both passage rates ($EDInsP_6$/fraction B2 ≥ 0.965; $EDInsP_6$/fraction C ≥ −0.932).

DISCUSSION

Influences of the processing details

As expected, increasing the residence time in the DT and additional heat treatment of a blend of the four examined RSM shifted the proportions of protein fractions from fraction B1 and B2 mainly to fraction C resulting in a respective decrease of EDCP. This is consistent with other studies using RSM[9,27] or flaxseed.[28] Physical methods such as dry and moist heating are commonly used to increase the content of RUP in rapeseed and RSM.[29] Structure and chemical profile of proteins are changed by heat exposure, which can affect the access of digestive enzymes and, thus, the availability of the protein.[28] However, heating can also lead to the formation of linkages resistant to enzyme attack, shifting the protein to the indigestible protein fraction C.[30] It is therefore important to find the optimum balance between heat denaturation and damage through the Maillard reaction.[30] It has been suggested that the point of maximum insoluble but still digestible protein represents the theoretical optimum heat input.[30] For the examined RSM this applies to RSM48 and RSM64, where the degradable fractions B2 and B3 accounted for 650 and 660 g kg^{-1} CP and the soluble fraction B1 was 160 and 140 g kg^{-1} CP, respectively. Although prolonging the residence time in the DT beyond 64 min decreased fraction B1 further (100 to 75 g kg^{-1} CP), this only resulted in higher amounts of fraction C (190 to 230 g kg^{-1} CP). As Maillard proteins contribute to fraction C[31] this indicates that the hot steam treatment for a period longer than 64 min may have stimulated Maillard reactions. The results of the chemical protein fractionation were reflected in the values of the *in situ* ruminal incubation. Only small differences in the degradation rate of CP and EDCP were observed between RSM48 and RSM64, these values decreased more distinctly for all other heat treatments (Table 2). The higher contents of RUP in RSM76, RSM93, and RSMover might be desirable if the ruminally undegraded protein is available for degradation and absorption in the intestine. However, Maillard proteins are not only highly resistant to ruminal degradation – they are also not digestible postruminally.[22]

Moderate heat treatments of RSM (125 °C for up to 30 min) increased fraction C only 1.4-fold but 8.8-fold when heated for the same period of time at 145 °C.[32] In the same study, RUP was increased for both temperatures but its intestinal disappearance was 30 percentage points lower for the 145 °C treatments compared to the 125 °C treatments. In the present study, we did not analyze the intestinal digestibility of the RUP but probably it was low for RSM76, RSM93, and RSMover due to the high percentage of fraction C. This assumption is supported by data obtained with the same batches of feed investigated in ileum-cannulated pigs.[33] In that study, the prececal digestibility of CP and most amino acids decreased in a linear manner with increasing residence times in the DT (CP: 660, 650, 620, 600, 620 g kg^{-1}; lysine: 640, 620, 590, 540, 550 g kg^{-1} for RSM48, RSM64, RSM76, RSM93, and RSMover, respectively).

Increasing the residence time in the DT changed the pattern of InsPs in the way that lower concentrations of $InsP_6$ and higher concentrations of mainly $InsP_5$- and $InsP_4$-isomers were analyzed (Table 1). Processed feeds commonly show higher concentrations of lower inositol phosphates[34] and degradation of $InsP_6$ caused by high temperature and pressure has been observed previously in RSM.[10,35] However, $InsP_6$ was the most abundant inositol phosphate in the bag residues throughout all incubation times for all RSM and no accumulation was observed for lower InsPs. This observation is consistent with results from previous studies using different feedstuffs where hardly any lower InsPs were detected in the bag residues after ruminal incubation.[10,21] Not even the harsh heat treatment of the RSM led to an accumulation of lower InsPs during ruminal incubation, so it can be assumed that in ruminants the crucial step in $InsP_6$ degradation is the cleavage of the first phosphate group irrespective of the feed or its pretreatment.

Generally, the disappearance of the ruminal $InsP_6$ and thus the release of $InsP_6$-P from RSM was remarkable, even at high ruminal passage rates, if the processing conditions were moderate (ED_8InsP_6: RSM48 650 g kg^{-1} $InsP_6$, RSM64 635 g kg^{-1} $InsP_6$). However, with increasing residence time in the DT and the associated formation of Maillard proteins, $EDInsP_6$ was affected similarly

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to EDCP. The reduction of $EDInsP_6$ showed almost identical statistical differences between the heating steps as observed for EDCP, reflecting the close relationship between protein and phytate in rapeseed. Phytate is located in globoids inside the protein storage vacuole of rapeseed and forms tight associations with the protein inside the globoids.[11] As the association between protein and $InsP_6$ remains unchanged during the production process of RSM[12] heat treatment of RSM affects $EDInsP_6$ to a remarkably similar extent as EDCP. Thus, it can be assumed that when the variation in rumen-degraded protein of RSM is ascribed to different processing conditions in oilseed plants,[6,7] this also affects rumen-degradable $InsP_6$ in RSM, explaining the considerable variation in the $EDInsP_6$ of commercial RSM (ED_8InsP_6: 590 g kg^{-1} $InsP_6$ (4, 5), 510 g kg^{-1} $InsP_6$ (own data, unpublished), 480 g kg^{-1} $InsP_6$[21]).

Contrary to RUP, ruminally undegraded $InsP_6$ is probably only of minor nutritional value for the animal. First, postruminal $InsP_6$ degradation is relatively low compared to ruminal degradation (10 to 230 versus 650 to 950 g kg^{-1} $InsP_{6)}$.[16,17,36] Second, postruminal $InsP_6$ degradation mainly occurs in the large intestine through microbial activity.[16,36] Although P can be absorbed in the large intestine to a small extent, the main site of intestinal absorption of P is located between the proximal duodenum and terminal ileum.[15] Thus, an increased ileal $InsP_6$ flow, as was observed after the inclusion of heat treated RSM in diets for sheep,[37] probably renders the postruminally released P mostly unavailable for the animal and contributes mainly to an increased fecal P excretion. This may lead to conflicting aims in RSM production when RUP is increased to improve postruminal protein supply of dairy cows in conditions when the overall P supply is low. However, the P concentration in RSM-containing diets often exceeds the P requirement of the cows, which makes it irrelevant whether part of the P is excreted in the form of $InsP_6$ or other forms.

Correlation between crude protein and phytate degradation

The close relation between CP and $InsP_6$ in RSM might be useful to predict $EDInsP_6$ from EDCP or protein fractions. Due to the significant influence of processing conditions on $EDInsP_6$ shown in this study, values for the ruminal release of $InsP_6$-P determined for one commercial RSM can hardly be transferred to another. Thus, $EDInsP_6$ should be considered in diet formulation on a regular basis to ensure the more precise calculation of dietary P supply. Figure 3 suggests that ED_8InsP_6 can be predicted from ED_8CP using a linear model. The close linear relationship between EDCP and $EDInsP_6$ is independent from the assumed ruminal outflow as the linear model for ED_5CP and ED_5InsP_6 also showed a high coefficient of determination ($R^2 = 0.95$). For this approach, however, *in situ* ruminal incubation of the feeds and analysis of N in bag residues is still necessary to obtain EDCP values to insert into the equation. An easier approach might be to use the correlations between the protein fractions and $EDInsP_6$ shown in Table 4 to establish a model to estimate $EDInsP_6$ routinely after protein fractionation. We derived high model quality when we predicted $EDInsP_6$ and *c* for the six rapeseed feeds described here using their protein fractions (ED_5InsP_6 (g kg^{-1}): -2336-0.38*B1 + 6.45*B2 + 0.74*C; $R^2 = 0.96$; RMSE = 26.3; ED_8InsP_6 (g kg^{-1}): -3088-0.41*B1 + 7.77*B2 + 1.08*C; $R^2 = 0.95$; RMSE = 31.4; *c* (% h^{-1}): 558–0.58*B1-0.49*B2-0.64*B3-0.75*C; $R^2 = 0.99$; RMSE = 0.21). Both approaches seem promising but more data are still required to improve the quality of the estimation.

CONCLUSIONS

The processing conditions of RSM influence not only the ruminal degradation of protein but also that of $InsP_6$. Hence, processing of RSM for high RUP content can reduce the digestible P supply for the animal. However, when moderate processing conditions are applied to maximize intestinal availability of RUP a sufficient amount of $InsP_6$-P seems to be intestinally available for the animal. Processing conditions in oilseed plants differ widely, so a regular evaluation of RSM in respect to $EDInsP_6$ based on EDCP or protein fractions is suggested for the precise calculation of the dietary digestible P supply.

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