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Ensiling and thermic treatment effects on ruminal carbohydrate fermentation and post-ruminal crude protein concentration in partial-crop peas and faba beans

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RUMINANTS

Abstract

This study was conducted to examine effects of ensiling, toasting or ensiling plus toasting in partial-crop field peas and faba beans harvested each with 375 g dry matter/kg (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) scale 79 and 81, respectively) on gas production and post-ruminal crude protein (PRCP) concentration by *in vitro* incubation in ruminal fluid batch-cultures. The silages made from partial-crop field peas and faba beans (Rostock Model Silages) had a pH of 4.3 and 4.6, respectively, and were not typically lactic acid dominated. The silages remained stable after opening for 100 h (peas) and 168 h (faba beans). Toasting was simulated in a drying oven at 160 °C for 60 min. Post-incubation pH and gas accumulation profiles were little affected by treatment. Ensiling did not alter effective PRCP. Toasting and ensiling plus toasting increased effective PRCP up to 25 and 20%-points in peas and up to 35 and 11%-points in faba beans, respectively. Ensiling increased non-protein nitrogen and soluble protein concentration, whilst toasting decreased soluble protein. Significant correlations existed between protein fraction B3 (neutral detergent-insoluble protein) and effective PRCP ($r \geq 0.84$; $P<0.05$) and fraction C (acid detergent-insoluble protein) and effective PRCP ($r \geq 0.79$; $P<0.05$). Ensiling and toasting both decreased arginine and lysine levels. It was concluded that partial-crop peas and faba beans with BBCH 79 and 81, respectively, can provide readily available nutrients and high-quality fibre in the residual plant. However, preserving by ensiling required balance between the reduction of non-protein nitrogen and fermentability characteristics. Toasting reduced protein solubility and increased PRCP, but it was not clear if PRCP was usable for ruminants or was partially bound into Maillard polymers.

Keywords: beans, peas, gas production, protein, ruminants

1. Introduction

Typically, feeds for dairy cows and other ruminant livestock include field peas (*Pisum sativum*) and faba beans (*Vicia faba*), which are rich in starch and protein. Concentrations of 478 and 422 g/kg dry matter (DM) starch and 251 and 298 g/kg DM crude protein (CP), respectively, are stated in German feed value tables (DLG, 1997). This makes them suitable for at least partial substitution of traditional protein feeds, such as soybean meal and other oilseeds in the ration (Corbett *et al.*, 1995; Tufarelli *et al.*, 2012). Interest in using indigenous legumes as alternative protein for livestock is increasing globally, due to prices and availability (Khan *et al.*, 2016). This would support feed production at a local level and enable better control of product quality (e.g. non-genetically modified feeds). Field peas and faba beans are likewise used as whole-crop forages up to the late flowering period or early pod development (McKnight and MacLeod, 1977; Mustafa and Seguin, 2003; Salawu *et al.*, 2002), which

are rich in neutral detergent fibre (aNDFom), at 410 and 457 g/kg DM, respectively, low in starch, at 73 and 29 g/kg DM, respectively, but with useful CP, at 177 and 200 g/kg DM, respectively (Mustafa and Seguin, 2003). Even pea or bean straw cut at full grain maturity still contained 151 or 86 g CP/kg DM, respectively (Alkhtib *et al.*, 2016; Borreani *et al.*, 2007). Partial-crop field peas or faba beans are cut beneath the lowest pods to fully utilise starch, protein and other nutrients, but to limit fibre content. Nutrient storage in legumes is usually complete at green ripening or dough-ripe stage and harvesting, at that point in time, produces feed rich in starch, protein and fibre.

Peas and faba beans contain large amounts of soluble protein (SP), which undergoes rapid degradation in the rumen (Vaga *et al.*, 2017; Yu *et al.*, 2002). Degradation rates are slower for starch than protein, and consequently, available energy from starch fermentation may restrict the synthesis of microbial protein from feed nitrogen (Focant *et al.*, 1990). Strategies to increase rumen-undegraded protein (RUP) from feed and maintain amino acid flow to the small intestines are, therefore, required.

Ensiling peas and faba beans is a common method of conservation and stockpiling, but this may increase non-protein nitrogen (NPN) and SP concentrations (Mustafa and Seguin, 2003). In order to control NPN formation and reduce SP in the rumen, harvesting at a later stage of plant maturity could be an option and thermic processing might be another (Aufrère *et al.*, 2001; Goelema *et al.*, 1998). Silages made at the beginning of the ripening stage have protein that is more concentrated in the beans or peas, making it less accessible for microbial proteases (Cavallarin *et al.*, 2007; Fraser *et al.*, 2001) and proteolysis and NPN formation can be significantly reduced (Cavallarin *et al.*, 2007).

Thermic processing by roasting, micronisation, autoclaving, toasting, extrusion or expansion has been shown to increase RUP and resistant starch in legumes (Bachmann *et al.*, 2020; Goelema *et al.*, 1998; Ljøkjel *et al.*, 2003; Masoero *et al.*, 2005; Mustafa *et al.*, 1998; Vaga *et al.*, 2017; Yu *et al.*, 2002). Post-ruminal CP (PRCP), which is the sum of RUP and protein synthetised by rumen microorganisms, will then increase, provided that the treatment has not impaired microbial protein production (Bachmann *et al.*, 2020). According to published papers, information on effects of thermic treatment in matured whole-crop or partial-crop peas and faba beans or in silages made from these crops is limited. Hence, the objective of the current study was to examine the effects of ensiling, toasting or the combination of both in partial-crop field peas and faba beans on gas production as a measure of ruminal carbohydrate fermentation and on PRCP concentration throughout 48 h of *in vitro* incubation in ruminal fluid batch-cultures.

2. Materials and methods

Ethical statement

The animals used in this study were kept and cared for by the Research Centre for Agricultural and Nutritional Sciences, Martin Luther University Halle-Wittenberg, 06193 Wettin-Löbejün (Germany) and the protocol was approved by Saxony-Anhalt Federal Administration Authority (approval no. 42505-3-813).

Donor animals and basal feeding

Rumen fluid was taken from two adult wethers which were randomly selected out of a group of six animals. Sheep had free access to tap water and meadow hay was offered *ad libitum* and supplemented with 200 g pelleted concentrate (IBEKA® PANTO Schäferstolz, HL Hamburger Leistungsfutter GmbH, Hamburg, Germany) and 10 g mineral feed (basu-kraft® Top-Mineral, BASU Heimtierspezialitäten GmbH, Bad Sulza, Germany) per animal per day. Nutrient concentrations in the feed (hay/concentrate) was as follows: 942/913 g DM/kg, 76/71 g crude ash (CA), 100/178 g CP, 13/31 g acid ether extract (AEE), 323/41 g crude fibre (CF), 671/135 g aNDFom, 387/58 g acid detergent fibre (ADFom) and 48/8 g acid detergent lignin/kg DM.

Substrates and treatments

The field pea cv. Astronaute and the faba bean cv. Tiffany (Norddeutsche Pflanzenzucht Hans-Georg Lembke KG, Holtsee, Germany) were grown in 2019 in Köllitsch, Saxony (51° 30' 11.304' N, 13° 6' 46.638' E). The plants were harvested by hand at approximately 10 cm height, and at 375 g DM/kg, which corresponded with stage 79 and 81 on the phenological BBCH scale, respectively. This is the point where approximately 10 to 30% of pods are fully ripened (Lancashire *et al.*, 1991).

Native material (i.e. as harvested) was chopped by hand to 8 to 15 mm particle size and ensiled in vacuum-sealed polyethylene bags (Rostock Model Silages; Hoedtke and Zeyner, 2011) for 60 d (three replicates per treatment as 1.5 kg samples) using Josilac® classic (Josera GmbH & Co. KG, Kleinheubach, Germany) as ensiling agent. This commercial preparation included *Lactobacillus plantarum* LSI NCIMB 30083 1k20736 and L256 NCIMB 30084 1k20737 as well as *Pediococcus acidilactici* P11 DSM 23689 1k1011 and P6 DSM 23688 1k1010. A total of 1.0×10^{11} colony forming units per gram fresh weight was applied from the bacterial strains. The silages were stored at approximately 25 °C. They had a pH of 4.3 and 4.6, 76.0 and 70.7 g acetic acid, and contained 9.2 and 20.8 g ethanol/kg DM, respectively. In both legume silages, the concentration of lactic acid was less than 0.1%. Propionic acid, i- and n-butyric acid, i- and

n-valeric acid, 1,2-propanediol and 1-propanol were only present in trace amounts. In silage, NH_3 was 4.6% (field peas) and 3.1% of total nitrogen (faba beans). The silages were stable under aerobic storage conditions for 100 and 168 h with a pH of 7.0 and 4.5 in field peas and faba beans, respectively. Aerobic stability was tested using the method of Honig (1990).

Both native and ensiled field peas and faba beans were toasted in a drying oven at 160 °C for 60 min using 1 kg of the material (i.e. 5 cm layer height of loosely packed material in a 21×33 cm aluminium shell). The material was mixed by hand every 10 min. Data regarding the chemical composition of field peas and faba beans, protein fractions and amino acid concentrations can be found in Supplementary Table S1, S2 and S3, respectively.

In vitro incubation

Batch-culture incubation comprised 20 runs, which were four runs per incubation time (i.e. four replicates from 2, 4, 8, 24 and 48 h) and each run comprised the full set of substrates/treatments as duplicate measures. Ruminal fluid samples were mixed and filtered through two layers of cheesecloth and a buffer/nutrient solution was added 1:2 (*v*/*v*) under a carbon dioxide flush. Buffer composition and preparation followed prescriptions of the Association of German Agricultural Analytic and Research Institutes (VDLUFA, 2012, method no. 25.1) and Edmunds *et al.* (2012). The rumen fluid had a pH of 6.5±0.11, a redox potential of -288±14.0 mV and temperature 36±0.83 °C. The inoculum (after buffer addition) had a pH of 6.7±0.037 and a redox potential of -245±34.5 mV. A total of 0.2 g of pulverised samples and 30 ml inoculum were added to a glass fermenter bottle to give 136±2.68 ml total volume and 106 ml headspace volume and were incubated at 39 °C in 80 rpm, shaking water baths.

Fermentation profiles

Gas production during the 48 h of incubation was assessed using the ANKOM RF Gas Production System (ANKOM Technology, Macedon, NY, USA) as described by Bachmann *et al.* (2020). Before incubation started, oxygen was purged from each fermenter bottle by venting with argon. The pressure of headspace gas accumulating in the fermenter bottles was detected and cumulatively recorded using the following settings: 1.5 psi threshold for automatic gas release, 150 ms valve open time, and 1 min recording interval. The gas pressures were blank corrected (using the mean of the four blank controls per run) and converted to ml of gas produced using the Ideal Gas Law and Avogadro's Law.

Post-ruminal crude protein

Samples were taken from the liquid phase of the fermenter bottles after 2, 4, 8, 24 and 48 h of incubation and NH_3-N was analysed, as described by Bachmann *et al.* (2020). Briefly, the fermenter bottles were immediately placed in ice water to stop microbial activity and the entire contents of the fermenters were rinsed into distillation tubes using 2×30 ml distilled water. Then 4 ml NaOH (1 mol/l) was added to transform ammonium ions into NH_3, and NH_3-N was analysed by distillation and titration using a FOSS 8400 Kjeltec Analyser (FOSS GmbH, Rellingen, Germany). No additional lye or water was added. The PRCP concentration was calculated as follows:

PRCP (g/kg DM) = ((NH_3-N (blank) + N (feed) – NH_3-N (sample)) × 6.25 × 100,000) / (200 × DM (feed))

where NH_3-N (blank) was NH_3-N measured in blanks (mg); N (feed) amount of nitrogen in 200 mg of feed sample, i.e. 200 mg × DM of sample (%) × CP of sample (%) / 6.25 / 10,000; NH_3-N (sample) was NH_3-N measured in the sample solution and DM (feed) was the DM concentration of the feed sample (%). The PRCP was expressed on a CP basis to consider differences in concentration that had been observed among the samples.

Chemical analyses

Prior to chemical analysis, samples of native and ensiled materials were pre-dried and, like the toasted and ensiled plus toasted materials, grounded to 1 mm sieve size using a laboratory sample mill. Concentrations of DM, CA, CP, AEE, starch, CF and detergent fibres were analysed in accordance with VDLUFA (2012, methods no. 3.1, 4.1.1, 5.1.1 B, 6.1.1, 6.5.1, 6.5.2, 6.5.3, 7.2.1 and 8.1). Neutral detergent fibre was determined after 1 h pre-treatment with heat stable amylase and was, as well as ADFom, expressed exclusive of residual ash. The proportion of nitrogen-free extract (NFE) was calculated as follows:

NFE = 1000 – CA – CP – AEE – CF

Gross energy (GE) was determined by bomb-calorimetry using a C7000 Oxygen Bomb Calorimeter (IKA® Werke, Staufen, Germany). Metabolizable energy (ME) was calculated using the equation for legume crops provided by the Society of Nutrition Physiology (GfE, 2017). Note that this equation is based on digestibility studies with alfalfa, red clover, white clover and sainfoin. Gas production was determined using the Hohenheim Gas Test (Menke *et al.*, 1979). The results should therefore be interpreted with caution. Protein fractions of the Cornell Net Carbohydrate and Protein System were determined using the method of Licitra *et al.* (1996). Within this, fraction A referred to NPN, fraction B1 to true protein (TP) that was soluble in borate-

phosphate buffer at around pH 6.7 but was precipitable, fraction B2 referred to neutral detergent-soluble TP (that which was insoluble in the buffer minus TP that was insoluble in neutral detergent), fraction B3 referred to TP that was insoluble in neutral detergent but soluble in acid detergent, and fraction C referred to TP that was insoluble in acid detergent. Residual nitrogen was determined using the Kjeldahl method (Kjeldahl, 1883). On that basis, TP and SP concentrations were calculated as follows:

TP = B1 + B2 + B3 + C, and SP = A + B1

Concentrations of amino acids were analysed after protein hydrolysis with hydrochloric acid using a Biochrom 30 Amino Acid Analyser fitted with PEEK-Sodium Prewash Column (100×4.6 mm) and PEEK-Oxidised Feedstuff Column (200×4.6 mm; Biochrom Ltd., Cambridge, UK) according to VDLUFA (2012, method no. 4.11.1). For detection of tryptophan, proteins were hydrolysed with phosphoric acid and hydrochloric acid. Tryptophan was analysed by liquid chromatography using an Agilent 1100 Series unit with ZORBAX Eclipse XDB-C8 column (150×4.6 mm, 5 µm; Agilent Technologies Inc., Santa Clara, CA, USA) according to the method described by Fontaine *et al.* (1998).

Statistical analysis

Statistical analysis was performed using SAS 9.4 software package (SAS Institute Inc., Cary, NC, USA). Prior to analysis of pH, gas production and PRCP data, fermenter bottles that showed gas leakage, defects or obvious measuring errors during incubation were excluded. The progression of pH during incubation was analysed by quadratic regression using the REG procedure and differences among incubation time points, crop species, or treatments were assessed using 95% confidence limits of the mean and 95% prediction limits. The gas production data set was scaled down to a 0.5 h resolution and non-linear regression was applied using the MODEL procedure and Gompertz function, according to Dhanoa *et al.* (2000). To assess differences among species or treatments, 95% confidence intervals were applied. For the PRCP data set, outliers were identified using boxplots and removed, being defined as observations more than three times of interquartile range. The PRCP concentration was displayed on a log time scale (ln(t)) according to H. Steingaß (personal communication) and the REG procedure was used to test whether linear, quadratic, or linear plus quadratic regression gave the best fit for PRCP progression. This was assessed using Mallows's Cp criterion (Mallows, 1973) and Akaike's information criterion (Akaike, 1974). Again, differences among incubation time points, crop species or treatments were assessed using 95% confidence limits for the mean and 95% prediction limits. The resulting regression equations were used to calculate effective PRCP concentration at assumed ruminal passage rates (Kp) of 0.02 (Kp2), 0.05 (Kp5) and 0.08/h (Kp8). For this, y was the effective PRCP concentration and x=3.91 at Kp2 (x = ln(100/2)), x=3.00 at Kp5 (x = ln(100/5)) and x=2.53 at Kp8 (x = ln(100/8)). For analysis of pH and gas production, four run replicates were available. For PRCP analysis, removal of outliers led to the following available replicates: two replicates in 2/40 cases, three replicates in 10/40 cases, and four replicates in 28/40 cases (i.e. eight substrates × five incubation times). Pearson correlation coefficients were calculated among TP fractions, arginine and lysine concentration, gas production kinetics and PRCP at Kp2, Kp5 and Kp8, respectively, using the CORR procedure.

3. Results and discussion

Fermentation profiles

Post-incubation pH profiles are illustrated in Supplementary Figure S1. The pH of the inoculum was pre-set to 6.7±0.037 and was equal for all fermenters. After 2 h of incubation, pH was 7.4±0.11 (7.3 to 7.7). The batch-culture medium was buffered by bicarbonate, phosphate and other agents added to or being present in ruminal fluid. Accumulated gases were regularly released from the fermenters, which lowered the partial pressure of carbon dioxide in the gaseous phase. This has been directly associated with increased efficiency of bicarbonate buffering and increase in pH (Dijkstra *et al.*, 2012; Kohn and Dunlap, 1998). Moreover, forage peas and faba beans (fresh and wilted) can have a relatively high buffering capacity (up to 710 mEq/kg DM; Pursiainen and Tuori, 2008; Rondahl *et al.*, 2011). However, the pH declined until 24 h in a curvilinear manner and was lowest at 7.1±0.22 (6.9 to 7.6); then, in most cases, the pH slightly increased until 48 h of incubation as fermentation activity declined. These alterations were not statistically significant and there was no difference found concerning crop species or treatment at the several incubation times.

A description of gas accumulation profiles for field peas and faba beans over 48 h of incubation is shown in Table 1. The measured gas production from partial-crop peas and faba beans ranged between the published values for legume grains (Azarfar *et al.*, 2008; Bachmann *et al.*, 2020; Pelagalli *et al.*, 2020) and hay (Bastida Garcia *et al.*, 2011). Consistent with the literature, more gas was produced from field peas than from faba beans. In partial-crop field peas, gas production was higher when ensiled or toasted (P<0.05). Toasting field pea silages decreased gas production (P<0.05). In peas, the time until one-third of the maximum amount of gas was formed was significantly delayed by all processing, and the time until another third formed was likewise delayed, except after ensiling (P<0.05). Fermentation of faba beans was, in general only slightly affected by ensiling or toasting. A faster production of gas was seen in faba bean silages and toasted silages (P<0.05). In peas and faba beans,

Table 1. Estimated model parameters of gas production kinetics of native, ensiled, toasted and ensiled plus toasted partial-crop field peas and faba beans in a batch-culture system using Gompertz non-linear regression function (Dhanoa *et al.*, 2000).[1]

Substrate	*a*	*b*	*c*	*b* + *c*	RMSE	R^2
Field peas						
Native	179 (179 180)	5.5 (5.4 5.6)	4.9 (4.7 5.0)	10.4	2.80	0.997
Ensiled	195 (194 196)	6.5 (6.3 6.6)	4.4 (4.3 4.6)	10.9	3.88	0.996
Toasted	190 (189 191)	6.6 (6.5 6.7)	5.8 (5.6 6.0)	12.4	3.52	0.996
Ensiled plus toasted	169 (168 170)	5.8 (5.7 6.0)	5.4 (5.2 5.6)	11.2	3.48	0.995
Faba beans						
Native	151 (150 152)	8.1 (8.0 8.2)	7.3 (7.1 7.5)	15.4	2.19	0.998
Ensiled	151 (150 152)	6.7 (6.5 6.8)	5.9 (5.7 6.2)	12.6	3.33	0.995
Toasted	156 (155 158)	8.1 (7.9 8.3)	7.5 (7.2 7.8)	15.6	3.74	0.994
Ensiled plus toasted	147 (146 147)	7.0 (6.9 7.1)	5.5 (5.3 5.7)	12.5	2.63	0.997

[1] *a* = asymptotic maximal gas production (ml/g dry matter); *b* = time (h) until which one-third of *a* is produced; *c* = time (h) between *b* and *b* + *c*, the time (h) until which 70% of *a* is produced; RMSE = root mean square error. Likelihood ratio 95% confidence intervals are given in brackets. Treatments are specified in the text.

hydrolysable starch is a main contributor to gas production in the batch-culture system. Using formed gas volume in relation to starch content allows only a rough estimate, since the proportion of hydrolysable starch was not known in this trial. However, the data revealed that, in peas, ensiling, toasting or applying both reduced gas production from 668 to 557 ml and 646 ml and 621 ml/g starch, respectively, which could have been due to binding of carbohydrates into Maillard polymers. Hofmann *et al.* (2020) have shown that the Maillard reaction occurs in pea grains following ensiling and toasting treatment. In relation to starch concentration, gas production in faba beans was increased after ensiling (from 808 to 878 ml/g starch) and decreased after toasting or ensiling plus toasting (from 808 to 746 ml and 631 ml/g starch, respectively). During ensiling, parts of starch and oligomeric sugars degrade to readily soluble substrates (Gefrom *et al.*, 2013) which are easily fermented by the microbes in the batch-culture. Hence, gas production is higher and the asymptote is reached earlier.

Post-ruminal crude protein

Measured and predicted concentrations of PRCP are displayed in Figure S2 on a log time scale over 48 h incubation. According to AIC and Mallow's *Cp*, a linear plus quadratic regression model best fitted PRCP progression. Significant differences among ln(2) to ln(48) incubation times were detected using 95% confidence and prediction limits (P<0.05). The predicted PRCP concentration was similar between ln(2) and ln(4), but decreased until ln(48) in native and ensiled peas and faba beans and ensiled plus toasted faba beans (P<0.05). In toasted peas and faba beans and ensiled plus toasted peas the maximum PRCP concentration was at ln(8), being nearly 1,000 g/kg CP or more. In toasted field peas, a considerable increase was found until ln(8) (P<0.05). Then, PRCP concentration rapidly declined (P<0.05).

At ln(4), ln(8) and ln(24), PRCP was markedly higher in toasted than in native or ensiled field peas (P<0.05). The toasted and ensiled plus toasted variants only differed at ln(4) (P<0.05). At ln(8), ln(24) and ln(48), PRCP was higher in ensiled plus toasted peas than in the native or ensiled counterparts (P<0.05). In faba beans, PRCP concentration was highest in the toasted variant starting from ln(8) (P<0.05). Treatment effects were not detected on the basis of 95% prediction limits.

Equations obtained from regression analysis were used to calculate effective PRCP at *K*p2, *K*p5 and *K*p8, as shown in Table 2. Effective PRCP concentration was higher in partial-crop faba beans than in field peas, and both were higher than data from grain and grain silages (Bachmann *et al.*, 2020). Ensiling did not distinctly alter effective PRCP in partial-crop peas and faba beans and this was similar in respective grain silages (Bachmann *et al.*, 2020). Toasting increased effective PRCP by 25%-points in peas and 35%-points in faba beans. The combination of ensiling and toasting increased effective PRCP by 20%-points in peas and 11%-points in faba beans.

In pods of peas and faba beans, there was no effect of toasting on PRCP concentration, but sometimes this decreased PRCP concentration on a CP basis (Bachmann *et al.*, 2020). At the same time, the proportion of RUP increased (more in peas than in faba beans), which meant that the proportion of microbial protein must have decreased (Bachmann *et al.*, 2020). Focant *et al.* (1990) reported that, in untreated peas, the degradation rate of starch was lower than protein. This means that ammonia concentration rapidly rises in the rumen (or in the *in vitro* fermenter), but there is not enough available energy for the microbes to utilise the nitrogen (Focant *et al.*, 1990). An increase in RUP alone did not restore the balance between starch and protein degradation and, thus, synthesis of microbial protein was limited by less available nitrogen

Table 2. Regression equations used for prediction of effective post-ruminal crude protein (PRCP) and effective PRCP concentration of native, ensiled, toasted and ensiled plus toasted partial-crop field peas and faba beans at assumed ruminal passage rates (*K*p) of 0.02 (*K*p2), 0.05 (*K*p5) and 0.08/h (*K*p8).[1]

Substrate	Regression equation	PRCP (g/kg crude protein)		
		*K*p2	*K*p5	*K*p8
Field peas				
Native	$y = 621.13 + 343.75 \times x - 107.65 \times x^2$	319	684	802
Ensiled	$y = 611.97 + 324.97 \times x - 102.36 \times x^2$	318	666	779
Toasted	$y = 557.11 + 632.73 \times x - 172.66 \times x^2$	391	901	1,053
Ensiled plus toasted	$y = 662.94 + 398.09 \times x - 111.23 \times x^2$	519	856	958
Faba beans				
Native	$y = 717.77 + 304.43 \times x - 101.46 \times x^2$	357	718	839
Ensiled	$y = 745.30 + 205.21 \times x - 75.029 \times x^2$	401	686	784
Toasted	$y = 707.25 + 392.44 \times x - 100.27 \times x^2$	709	982	1,058
Ensiled plus toasted	$y = 707.91 + 300.81 \times x - 92.868 \times x^2$	464	775	875

[1] x=3.91 at *K*p2 (x = ln(100/2)); x=3.00 at *K*p5 (x = ln(100/5)); x=2.53 at *K*p8 (x = ln(100/8)). Treatments are specified in the text.

and energy (Bachmann *et al.*, 2020). However, in partial-crop peas and faba beans, the concentration of PRCP increased in line with RUP concentration as a result of toasting and/or ensiling plus toasting, and was highest after 8 h of incubation. Microbial protein synthesis was therefore probably not inhibited, and the concentration of microbial protein remained largely unchanged in toasting treatments compared to the native materials. This finding suggested that, in the form of digestible non-starch polysaccharides (mostly glucose and xylose residues), the residual plant provides energy sources that are not provided by the grain alone (Åman and Graham, 1987).

Ensiling and the toasting clearly affected solubility of protein. Ensiling generally increased NPN and SP concentration, which confirmed previous studies with pea and faba bean grains (Bachmann *et al.*, 2020) and whole plants (Mustafa and Seguin, 2003). After toasting, the B1 protein fraction decreased by 32%-points (peas) and 11%-points (faba beans. For the insoluble fractions, B3 and C increased by 16%-points (peas) and 35%-points (faba beans) and 3%-points (peas) and 5%-points (faba beans), respectively (Table S2). Consequentially, the SP concentration decreased by 32%-points (peas) and 15%-points (faba beans) due to the effect of toasting (Table S2). Ensiling plus toasting mainly decreased the B1 fraction (in peas by 40%-points and in faba beans by 13%-points; Table S2). This, in turn, affected PRCP. Again, similar results were obtained from toasting of pea and faba bean grains (Bachmann *et al.*, 2020). Relevant correlations existed between protein fraction B3 and PRCP at *K*p2, *K*p5 and *K*p8, respectively ($r \geq 0.84$) and fraction C and PRCP ($r \geq 0.79$; Table 3), which indicated an increase

Table 3. Pearson correlation coefficients among true protein (TP) fractions, selected amino acids, parameters of gas production kinetics and post-ruminal crude protein at ruminal passage rates (*K*p) of 0.02 (*K*p2), 0.05 (*K*p5) and 0.08/h (*K*p8) in native partial-crop field peas and faba beans and corresponding treatments.[1]

	B1	B2	B3	C	Arg	Lys	*a*	*b* + *c*	*K*p2	*K*p5	*K*p8
B1	1	-0.09	-0.37	-0.42	0.35	0.42	0.42	-0.41	-0.61	-0.54	-0.49
B2		1	-0.26	-0.04	0.55	0.54	-0.41	0.30	-0.34	-0.17	-0.09
B3			1	0.97	0.23	-0.65	-0.13	0.64	0.85	0.89	0.84
C				1	0.34	-0.58	-0.32	0.77	0.82	0.84	0.79
Arg					1	0.31	-0.14	0.58	-0.16	0.04	0.11
Lys						1	0.20	-0.28	-0.83	-0.73	-0.64
a							1	-0.61	-0.42	-0.07	0.04
b + *c*								1	0.53	0.44	0.39
*K*p2									1	0.83	0.71
*K*p5										1	0.98
*K*p8											1

[1] B1 = buffer-soluble TP; B2 = neutral detergent-soluble TP; B3 = TP insoluble in neutral detergent, but soluble in acid detergent; C = TP insoluble in acid detergent. Arg = arginine; Lys = lysine. *a* = asymptotic maximal gas production; *b* + *c* = time until which 70% of *a* is produced. Significant correlations are highlighted (P<0.05).

in RUP as a result of toasting. Increased concentration of fraction C decelerated gas production (i.e. $b + c$ increased; r=0.77; Table 3). Moreover, most of the concentrations of amino acids declined as a result of ensiling plus toasting in peas and/or toasting without ensiling in faba beans (Table S3). Most prominent, however, were the changes found in arginine and lysine. The concentration of arginine decreased with ensiling (in peas from 11.4 to 8.5 g/kg DM, i.e. from 7.8 to 5.2 g/16 g nitrogen, and in faba beans from 14.4 to 8.4 g/kg DM, i.e. from 8.7 to 4.8 g/16 g nitrogen) and ensiling plus toasting (in peas from 11.4 to 6.4 g/kg DM, i.e. from 7.8 to 4.0 g/16 g nitrogen, and in faba beans from 14.4 to 9.8 g/kg DM, i.e. from 8.7 to 5.2 g/16 g nitrogen). Lysine decreased in peas after ensiling plus toasting (from 10.0 to 7.4 g/kg DM, i.e. from 6.8 to 4.6 g/16 g nitrogen) and, in faba beans, after toasting (from 10.0 to 7.5 g/kg DM, i.e. from 6.0 to 4.5 g/16 g nitrogen; Table S3). Lysine concentration on a DM basis was negatively correlated with B3 (r=-0.65), C (r=-0.58) and PRCP (r≤-0.64; Table 3). The increase of insoluble protein fractions, RUP and PRCP, deceleration of gas production and declining amino acid concentrations (especially for arginine and lysine) supported the assumption that carbohydrates and amino acids had been bound partly to Maillard reaction products. Hofmann *et al.* (2020) found that, in pea grains, ensiling increased lysine associated *N*-ε-2-furoylmethyl-l-lysine (furosine) and *N*-ε-(carboxymethyl) lysine (CML) and toasting increased furosine, CML, 2-amino-6-(2-formyl-5-hydroxymethyl-1-pyrrolyl)-hexanoic acid (pyrraline) and the arginine derivative *N*-δ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1). Concentrations of GE and ME generally increased following ensiling and toasting, which did not explain why toasting specifically increased PRCP.

4. Conclusions

At the stage of maturity for the peas and faba beans used in this study (i.e. BBCH 79 and 81, respectively), nutrient storage into the grain was largely complete. However, the residual plant still provided some portion of digestible non-starch polysaccharides. Harvesting partial-crop field peas or faba beans, therefore, combines readily available nutrients and a portion of high-quality fibre. The nutrient-to-fibre ratio can be controlled by selecting plants at a certain level of maturation. When ensiling, a balance needs to be struck between the reduction of NPN and ensilability. Drying/toasting preserved the crop and reduced the solubility of protein in the rumen and PRCP concentration increased. However, to what extent the extra PRCP was really available for ruminants or was partially bound by the formation of Maillard polymers, was not clear.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/JAAN2023.0001.

Table S1. Crude nutrient and detergent fibre concentrations of field pea and faba bean treatments.

Table S2. Crude protein fractions of field pea and faba bean treatments.

Table S3. Amino acid concentrations of field pea and faba bean treatments.

Figure S1. Measured and predicted pH-values during 48 h incubation of native, ensiled and thermally treated partial-crop field peas and faba beans in a ruminal fluid batch-culture system.

Figure S2. Measured and predicted concentrations of post-ruminal crude protein during 48 h incubation of native, ensiled and thermally treated partial-crop field peas and faba beans in a ruminal fluid batch-culture system on CP basis plotted on a log time scale (ln(t)).

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Conflict of interest

The authors declare no conflict of interest.

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