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Short communication

In vitro and *in vivo* analyses of the nutritive value of native and ensiled partial crop field peas

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ABSTRACT

This study was conducted to determine the effect of ensiling on the nutritive value of partial crop field peas. The field peas were harvested with approximately 500 g dry matter (DM) at a cutting height of 25 cm and ensiled without previous wilting or use of inoculants. *In vitro* gas production kinetics were determined in ruminal fluid batch cultures. The apparent total tract digestibility of crude nutrients, detergent fibers and gross energy and the content of metabolizable energy and net energy lactation of native and ensiled peas was determined in a digestibility trial with wethers. Microbial nitrogen efficiency was calculated on the basis of allantoin excreted via the urine. The pea silage was characterized by a low pH (4.1) and contained relevant quantities of lactic acid (56.3 g/kg DM) and acetic acid (16.8 g/kg DM). The proportions of crude protein, starch and nitrogen-free extracts were slightly increased in the silage, while the concentrations of fiber fractions decreased. Soluble protein was increased in the silage from 56% to 66% of crude protein due to increasing non-protein nitrogen concentrations. Gas production was higher in ensiled than in native peas ($P < 0.001$). Apparent digestibility of crude protein and acid ether extract increased in the silage by 3% and 10% points, respectively. Digestibility of crude fiber, neutral detergent fiber and acid detergent fiber decreased by 9%, 6% and 5% points, respectively ($P < 0.05$). The contents of metabolizable energy and net energy lactation increased by 0.5 and 0.3 MJ/kg DM, respectively ($P < 0.05$). The microbial nitrogen efficiency tended to be higher in animals that received ensiled partial crop peas ($P > 0.05$), but strongly depended on individual excretion quantities. In conclusion, ensiling of partial crop field peas with approximately 500 g DM/kg at harvest led to well conserved feed with increased concentrations of nutrients and content of energy.

Abbreviations: ADFom, acid detergent fiber expressed exclusive of residual ash; ADL, acid detergent lignin; AEE, acid ether extract; amP, absorbed microbial purines; aNDFom, amylase-treated neutral detergent fiber expressed exclusive of residual ash; BBCH, Biologische Bundesanstalt für Land- und Forstwirtschaft, Bundessortenamt und Chemische Industrie; CA, crude ash; CF, crude fiber; CP, crude protein; DM, dry matter; DOM, digestible organic matter; GE, gross energy; GfE, Society of Nutrition Physiology; ME, metabolizable energy; MN, microbial nitrogen; MNE, microbial nitrogen efficiency; NEL, net energy lactation; NFE, nitrogen-free extract; NPN, non-protein nitrogen; OM, organic matter; SCFA, short chain fatty acids; SP, soluble protein; TP, true protein; VDLUFA, Association of German Agricultural Analytic and Research Institutes.

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1. Introduction

Over the last years, there was growing interest in Europe to support the production of local protein resources. In addition to their beneficial effect on soil fertility when applied as cover- and/or catch-crop in cropping systems, grain legumes provide valuable protein when fed to farm animals (Watson et al., 2017). Partial crop field peas, cut directly beneath the lowest pod attachments, can be used as forage in ruminant feeding systems. They are enriched in protein and starch in comparison to the whole plant and therefore could substantially contribute to the energy supply of the animals. The concentration of nutrients and fiber of partial crop peas ranged between pea seeds and whole crop peas (Bachmann et al., 2022b).

Technical drying of pea forage would be a useful method for conservation as it avoids proteolytic processes that increase the proportion of non-protein nitrogen (NPN) and soluble protein (SP). It is, however, associated with high efforts and energy costs (Bachmann et al., 2022a) and is currently not feasible on-farm. Several studies have shown that ensiling of pea forages may provide preserved feed of good quality (Mustafa et al., 2002; Borreani et al., 2009). However, a major disadvantage of ensiling is proteolysis, which can cause a substantial increase in SP content. Proteolysis can be controlled by harvesting the plants at a proper stage of maturity or by wilting (Hartinger et al., 2019). The fermentation can further be stabilized by the use of inoculants (Bachmann et al., 2022b). These goals may not be compatible. Dry silage that is ensiled with a dry matter (DM) content of 500 g/kg or more can significantly reduce the risk of proteolysis but may not be a lactic acid silage in the classic sense. The material is rather preserved through dryness and air exclusion and is only supported by lactic acid fermentation to a limited extent (Bachmann et al., 2022b). In addition to undegraded feed protein, protein that is synthesized microbially in the rumen considerably contributes to the availability of post-ruminal crude protein and the supply of amino acids absorbable in the small intestines (Gresner et al., 2022).

The objectives of the present study were as follows: 1) to describe the nutritive value of native and ensiled partial crop field peas harvested with approximately 500 g DM/kg including nutrient, detergent fiber and energy contents, the proportion of SP and silage quality parameters; and 2) to determine the effect of ensiling on ruminal fermentation (gas production), microbial protein synthesis and total tract digestibility of nutrients, detergent fibers and energy.

2. Materials and methods

The field pea cultivar '*Astronaute*' was sown during April and May 2020 on a loamy alluvial soil with sand strip. It was harvested in July on the experimental fields near Köllitsch (Saxony, Germany) by direct cutting at approximately 25 cm height. A picture illustrating the cutting height of the partial crop peas can be found in Bachmann et al. (2022b). At the time of harvest, the peas ranged in Biologische Bundesanstalt für Land- und Forstwirtschaft, Bundessortenamt und Chemische Industrie (BBCH) growth stages between BBCH 81 and BBCH 85 (Meier, 2018). The content of DM ranged in the seeds between 600 and 700 g/kg and in the whole plant between 450 and 600 g/kg. Approximately 50% of the pulses were ripened. The pea seeds were cracked in the forage harvester and the plant material was chopped to a particle length of 8–15 mm. The partial crop peas were ensiled in round bales without use of inoculants for 69 days.

The *in vitro* incubations were carried out as described by Bachmann et al. (2022a). Ruminal fluid was taken alternately from two out of four adult rumen-cannulated wethers, which were kept and cared for by the Research Centre for Agricultural and Nutritional Sciences of the Martin Luther University Halle-Wittenberg and used with approval by the Saxony-Anhalt Federal Administration Authority (approval no. 42505-3-813). Tap water and meadow hay were offered ad libitum. Additionally, each animal received 200 g of a pelleted concentrate and 10 g of mineral feed per day. Specifications of the feeds are given by Bachmann et al. (2022a). The incubations comprised four consecutive runs (i.e., 4 replicates). Within each run, the test substrates were measured as duplicates. Ruminal fluid samples were mixed and filtered through two layers of cheesecloth before a buffer/nutrient solution was added at a ratio of 1:2 (*v/v*) under continuous carbon dioxide flush according to method no. 25.1 of the Association of German Agricultural Analytic and Research Institutes (VDLUFA, 2012). The ruminal fluid had a pH of 6.4 ± 0.086, a redox potential of − 271 ± 13.6 mV and a temperature of 35 ± 1.5 °C. The inoculum (after buffer addition) had a pH of 6.7 ± 0.0058 and a redox potential of − 263 ± 3.60 mV. A quantity of 0.2 g of substrate and 30 mL inoculum were added to glass bottles with an actual volume of 136 ± 2.68 mL (i.e., 106 mL headspace volume) and incubated at 39 °C in shaking water baths at 80 rpm for maximal 24 h. Prior to incubation, the test substrates were dried at 60 °C for 48 h, ground to pass a 1 mm screen of a sample mill, and then pulverized using a Retsch MM 400 ball mill (RETSCH GmbH, Haan, Germany) with 50 mL cup and a steel ball with a diameter of 2.5 cm at 25 vibrations/s. Accumulated headspace gas pressures were measured using the ANKOM RF Gas Production System (ANKOM Technology, Macedon, NY, USA). Before the incubation started, oxygen was purged out of each fermenter by venting the headspace with argon and the threshold for gas release was set to 1.5 psi. The gas pressures were corrected for blanks using a mean of four blanks within each run and converted to mL of gas produced.

For the digestibility trial, four wethers (Pomeranian coarsewool) were used, also kept and cared for by the Research Centre for Agricultural and Nutritional Sciences (approval no. 2–1524 MLU). All animals were clinically healthy and under regular veterinary supervision. Native or ensiled peas were administered at 1705 g/d on as fed basis divided into two equal meals. Additionally, the sheep received 10 g/d of mineral feed. Tap water was offered ad libitum. The feeding level of the sheep was 1.2 fold of the energy maintenance level recommended by the Society of Nutrition Physiology (GfE, 2001). The digestibility trial was conducted in two consecutive periods and followed the guidelines of the GfE (1991). The first period consisted of 21 d adaptation followed by 6 d of total collection of feces and urine, whereas the second period consisted of 14 d adaption and 6 d of total collection. Following this procedure, four sheep received either the native or the ensiled peas (i.e., two sheep received native and two other sheep received ensiled partial

crop peas in each period). The sheep were individually housed in metabolic cages. Feed residuals were recorded each morning. Feed residuals of two sheep were higher than 5% of the quantity offered. In accordance with the method prescription (GfE, 1991), these animals were excluded from further analysis. Therefore, for digestibility calculation, 3 replicates (i.e., animals) were available for each pea variant. During the total collection periods, the animals were fitted with harnesses to ensure reliable feces collection. The harnesses were emptied each morning immediately prior to the first meal. Defecation was quantified and an aliquot of 20% was taken each day. Aliquots of feces were merged to one sample for each sheep and each collection period. Feed and feces samples were frozen at − 20 °C to store for subsequent analyses. Urine samples from quantitative collections were taken during the collection periods (i.e., at 2 × 6 d), approximately four hours after the morning feeding. Plates underneath the metabolic cages for urine collections were thoroughly cleaned daily and the collection vessels were covered with cheesecloth. One sample of fresh urine was directly frozen at − 20 °C and used for the analysis of allantoin. Samples were thawed and combined to one bulk sample for each animal and each collection period and analyzed as described previously (Müller et al., 2021). Briefly, allantoin was measured by high performance liquid chromatography (1200/1260 infinity II Series; Agilent Technologies Inc., Santa Clara, CA, USA) on a 250 × 4 mm HyperClone ODS (C18) 120 Å column (Phenomenex Inc., Torrance, CA, USA) protected by a 4 × 3 mm C18 pre-column, constantly at 25 °C. Phosphate buffer (20 mM, pH 6.5) was used as mobile phase at a flow rate of 1 mL/min. Metabolites were detected at 230 nm wavelength (1260 Infinity II Multiple Wavelnegth Detector; Agilent Technologies Inc., Santa Clara, CA, USA).

The animals' body weights were recorded before adaptation as well as before and after the collection periods. The initial body weight was 66 ± 9.7 kg. The body weight was 64 ± 8.4 kg after the first adaptation period, 65 ± 7.4 kg after the first collection period, 67 ± 2.0 kg after the second adaptation period and 68 ± 1.7 kg at the end of the experiment.

Feces and urine samples were freeze dried and feed samples were dried at 60 °C for 48 h. Then, feces and feed samples were ground to pass a 1 mm screen of a standard laboratory sample mill. Dry matter, crude ash (CA), crude protein (CP), acid ether extract (AEE), crude fiber (CF) and detergent fiber concentrations were analyzed according to VDLUFA using methods no. 3.1, 4.1.1, 5.1.1 B, 6.1.1, 6.5.1, 6.5.2, 6.5.3, and 8.1 (VDLUFA, 2012). Starch was determined using the amyloglucosidase method (method no. 7.2.5) (VDLUFA, 2012). Neutral detergent fiber (aNDFom) was determined after pretreatment with a heat stable amylase. Neutral detergent fiber and acid detergent fiber (ADFom) were expressed exclusive of residual ash. Organic matter (OM) was calculated as OM = 1000 − CA, and the nitrogen-free extract (NFE) was calculated as NFE = OM − CP − AEE − CF. The protein fractions A (NPN), B1 (true protein, TP, which is soluble in borate-phosphate buffer at pH 6.7, but precipitable), B2 (TP, which is insoluble in the buffer minus TP, which is insoluble in neutral detergent), B3 (TP, which is insoluble in neutral detergent but soluble in acid detergent) and C (TP, which is insoluble in acid detergent) were determined according to the Cornell Net Carbohydrate and Protein System (Licitra et al., 1996). All measurements of nitrogen were performed using the Kjeldahl method. The protein fractions were used to calculate NPN (CP – TP), TP (B1 + B2 + B3 + C) and SP (A + B1) concentrations. Gross energy was determined in the feeds, feces and in urine by bomb calorimetry using a C7000 Oxygen Bomb Calorimeter (IKA® Werke, Staufen, Germany). Ammonia concentrations were determined according to the method of Conway and Byrne (1933). Lactic acid concentrations were determined using liquid chromatography following the internal method LKS FMUAA 166 (the laboratory was accredited according to DIN EN ISO/IEC 17025:2018). The short chain fatty acids (SCFA) produced during fermentation of pea silages were determined after aqueous extraction by gas chromatography using a Shimadzu GC2010 (Shimadzu Corp., Kyoto, Japan) fitted with a flame ionization detector. Analytes were separated on an SGE BP21 column (30 m × 0.53 mm × 0.5 μm) (Trajan Scientific and Medical, Ringwood, Australia). The extracts were centrifuged at 2000 × *g* before the supernatant was injected on-column. The following settings were used for detection of SCFA: 0.5 μL injection volume, 180 °C injection temperature, constant pressure of 22.7 kPa (i.e., 29.7 cm/s linear velocity and 3.64 mL/min column flow), 85 °C initial oven temperature, which was raised up by 8 °C/min to 200 °C and held for 6 min, and 200 °C detection temperature. Helium was the carrier and the make-up gas. The concentration of the target analytes was determined on the basis of an external standard calibration. Amino acids were determined according VDLUFA method 4.11.1. Briefly, feed samples were ground to pass a 0.5 mm screen of a sample mill. A quantity of 0.25 g was weighed in a glass beaker and incubated with 5 mL of oxidation solution (0.5 mL of hydrogen peroxide, 4.5 mL phenol-containing formic acid and 0.25 mg phenol) for 24 h at 0 °C. The oxidation was stopped with 0.9 g sodium metabisulfite. Then 50 mL of hydrolyzation mixture (492 mL 6 N hydrochloric acid and 1 g phenol in 1000 mL water) was added and incubated for 24 h at 110 °C. The solution was cooled down in an ice bath and transferred into buffer solution (19.6 mg trisodium citrate, 5 mL thiodiglycol, 1 g phenol and 16.5 mL 6 N hydrochloric acid in 1000 mL water at pH 2.2) and 40 mL 2 N sodium hydroxide in a beaker glass. The pH was adjusted with 2 N sodium hydroxide to 2.2 and the mixture was transferred with the buffer solution into a 200 mL volumetric piston. Two mL of internal standard was pipetted, filled up with buffer solution, shaken and 50–100 mL were filtered. The mixture was controlled to maintain the pH 2.2. Then, aliquots of 50 μL were analyzed using a Biochrom 30 Amino Acid Analyser (Biochrom Ltd., Cambridge, UK) connected with a Midas 830 autosampler (Spark Holland, Emmen, The Netherlands). Proline was measured at 440 nm and all other amino acids at 570 nm wavelengths.

Statistical analysis was performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Crude nutrient, amino acid and detergent fiber concentrations and GE content as well as the protein fractions were considered as descriptive data and did not undergo any statistical evaluation. For the analysis of gas production, all units in which gas leakage, defects or measuring errors were detected, were excluded (5/8 and 8/8 units remained for analysis for native and ensiled peas, respectively). Then, non-linear regression analysis was performed using the Gompertz function (Dhanoa et al., 2000) in the form $y(t)=a \times exp(-exp((b-t)/c))$. The model parameters a (the asymptotic maximal gas production), b (the time until which one third of a is produced), $b + c$ (the time until which 70% of a is produced) and c (the time between b and $b + c$) were estimated with the NLMIXED procedure and compared between the treatments (i.e., native and ensiled peas). Apparent digestibility coefficients of OM, CP, AEE, CF, aNDFom, ADFom, NFE, starch and GE were calculated as the difference between intake and fecal output divided by the intake on a daily basis for each individual. Metabolizable energy (ME) and net energy lactation (NEL) were calculated based on the apparent tract digestibility coefficients according to GfE (2001). To compare

the digestibility coefficients as well as ME and NEL contents between the two treatments, the pooled t-test was used after confirming homogeneity of sample variances using the folded F-test. Concentrations of microbial nitrogen (MN) were calculated according to Chen et al. (1992) using the following equation: MN (g/d) = 0.727 × amP, where amP is the sum of absorbed microbial purines. The amP was calculated according to Chen et al. (1990) using the Newton-Rhapson iteration process with 200 iterations, in the present case solely from allantoin excretion via the urine. Microbial nitrogen efficiency (MNE) was calculated according to Chen et al. (1992) related to the daily quantity of ingested digestible organic matter (DOM): MNE (g MN/kg ingested DOM) = MN/(0.65 × DOM intake). For comparison of MN and MNE between the treatments, the pooled t-test was used as well. The significance level for all statistical tests was $P < 0.05$.

3. Results

Fermentation characteristics of ensiled partial crop peas are shown in Table 1. The silage was characterized by a typically low pH and distinct lactic acid concentration. The concentrations of i- or n-butyric, valeric and caproic acid were below 0.05 g/kg DM and thus negligible.

The concentrations of crude nutrients, detergent fibers and GE are summarized in Table 2. Ensiling slightly increased CP and decreased detergent fiber concentrations. Furthermore, there were no differences observed between native and ensiled partial crop peas.

The ensiled peas had a higher concentration of SP and a lower concentration of TP. The protein fractions B3 and C remained unaffected.

The concentrations of amino acids were higher in native than in ensiled field peas (Table 4). Primarily, the concentrations of arginine and tryptophan were lower in ensiled partial crop peas.

The volume of gas produced after 24 h of incubation in the batch cultures was 23% points higher in ensiled than in native partial crop field peas ($P < 0.001$; Table 5). Ensiling decelerated *in vitro* ruminal fermentation ($P < 0.001$; Table 5).

The quantity of MN and MNE varied from 1.2–14.8 g/d and 1.3–17.8 g MN/kg ingested DOM, respectively. Calculated MN and MNE in ensiled peas were on average 1.5-fold and 1.7-fold higher than in native peas, respectively (Table 6). The content of GE in urine was similar in both pea treatments.

Apparent total tract digestibility coefficients ranged from 0.32–0.97 and differed between native and ensiled partial crop field peas in case of CF, aNDFom and ADFom ($P < 0.05$; Table 7). The contents of ME and NEL were higher in ensiled than in native peas ($P < 0.05$; Table 7).

4. Discussion

Partial crop field peas may provide a balance among protein, energy and fiber supply (Bachmann et al., 2022a). During growth and plant maturation, the proportion of stems and leaves decrease and that of the pods increase to approximately 20% of DM 12 weeks after sowing and 40% of DM 14 weeks after sowing (Fraser et al., 2001). Especially starch and protein are then enriched in the grains and cell wall components increase in vegetative parts of the plant (Åman and Graham, 1987). The concentrations of crude nutrients, detergent fibers and amino acids of native and ensiled partial crop field peas were similar to those published by Bachmann et al. (2022b). The concentration of starch, however, was lower than recent data suggested (268 and 350 g starch/kg DM in native and ensiled partial crop peas, respectively) (Bachmann et al., 2023). The content of ME and NEL in native and ensiled partial crop peas were above values given for corn- and grass silages (DLG, 1997).

In most cases, field peas are harvested as whole crops with DM contents below 300 g/kg. Wilting the crops and/or inoculation with lactic acid bacteria or other ensiling agents is in the most cases necessary to realize stable lactic acid based silages (Borreani et al., 2009). Proteolysis during ensiling is, however, still a problem, because of increasing SP and decreasing post-ruminal crude protein by proteolytic activity of microbial and plant enzymes. Proteolysis can be minimized by ensiling with higher DM contents of harvested material (350–400 g/kg) (Fijałkowska et al., 2015). The partial crop field peas used in this study were harvested with 472 g DM/kg. The peas were ensiled without previous wilting and use of inoculants. Borreani et al. (2009) observed with increasing DM content in unwilted and uninoculated whole crop pea silages, low levels of lactic acid and acetic acid. This indicates depressed fermentation activity. The current pea silages had lower pH and higher concentrations of lactic acid and acetic acid than those described by Borreani et al. (2009). This was probably due to a higher proportion of fermentable carbohydrates in the partial crops. As reported by Bachmann et al. (2022b), a distinct natural stock of lactic acid bacteria develops during maturation of the plant and they may contribute to stable silages even without the use of artificial inoculants. A more detailed comparison of the fermentation characteristics with literature data is not possible, because most silages were made after wilting for 6–96 h and application of bacterial or acid-based inoculants (Fraser et al., 2001; Mustafa et al., 2002; Mustafa and Seguin, 2003; Borreani et al., 2009; Rondahl et al., 2011).

Table 1
Fermentation characteristics of ensiled partial crop field peas.

pH	Lactic acid	Acetic acid	Propionic acid	NH_3-N
4.08	56.3	16.8	0.04	1.5

Lactic acid, acetic acid and propionic acid are given in g/kg DM.
NH_3-N is given in g/kg nitrogen.

Table 2
Concentrations of crude nutrients and detergent fibers and content of energy in native and ensiled partial crop field peas.

Treatment	DM	OM	CA	CP	AEE	CF	aNDFom	ADFom	ADL	Starch	NFE	GE	ME	NEL
Native	472	920	80	114	10	286	375	303	40	119	511	16.4	11.6	7.3
Ensiled	544	940	60	152	13	213	312	257	30	131	563	17.1	12.1	7.6

ADFom, acid detergent fiber expressed exclusive of residual ash; ADL, acid detergent lignin; AEE, acid ether extract; aNDFom, neutral detergent fiber treated with amylase and expressed exclusive of residual ash; CA, crude ash; CF, crude fiber; CP, crude protein; DM, dry matter; GE, gross energy; ME, metabolizable energy; NEL, net energy lactation; NFE, nitrogen-free extract; OM, organic matter.
DM is given in g/kg, crude nutrients, detergent fibers and starch are given in g/kg DM, and GE, ME and NEL are given in MJ/kg DM.

Table 3
Concentrations of crude protein, soluble protein and protein fractions in native and ensiled partial crop field peas.

Treatment	SP	TP	A	B1	B2	B3	C
Native	56	110	16	40	38	2	4
Ensiled	66	79	44	22	29	1	4

A, non-protein nitrogen; B1, buffer-soluble TP; B2, buffer-insoluble TP minus TP insoluble in neutral detergent; B3, TP insoluble in neutral detergent but soluble in acid detergent; C, TP insoluble in acid detergent; SP, soluble protein; TP, true protein.
A, B1, B2, B3, C and SP are given in % of crude protein.
Concentration of TP is given in g/kg dry matter.

Ensiled peas showed a significantly higher gas production *in vitro* than the native peas, but it took more time to achieve this.

In ensiled partial crop field peas, OM digestibility was generally higher than found previously in ensiled whole crop field peas (Salawu et al., 2002). Digestibility of protein increased as a result of higher concentrations of SP due to proteolysis during fermentation (Mustafa et al., 2002; Mustafa and Seguin, 2003). The concentrations of amino acids reported in the present study were similar to those given by Bachmann et al. (2022b). The decrease of the concentration of amino acids in ensiled peas, could be attributed to microbial degradation and proteolysis during ensiling (Cavallarin et al., 2006; Fijałkowska et al., 2015). Digestibility of CF and detergent fibers significantly decreased in ensiled field peas. This also indicates that during ensiling, hydrolyzation of potentially degradable fiber fractions leads to an enrichment of the lesser degradable fractions (Peyrat et al., 2014).

Estimation of the daily MN supply based on excreted purine derivatives provides a simple and non-invasive method (Chen et al., 1992). According to Tas and Susenbeth (2007), allantoin was used as the sole biomarker for the calculation of MN and MNE. The MN and MNE values were not corrected for appearance of endogenous purines, because reliable estimations were not possible (Chen et al., 1992; Tas and Susenbeth, 2007). As pointed out by previous studies (Balcells et al., 1991; Balcells et al., 1993; Tas and Susenbeth, 2007), allantoin excretion largely varies among animals fed the same diet due to nucleic acids in feed, their intestinal digestibility and endogenous release of purines (Tas and Susenbeth, 2007). It is noticeable that in the present study animal no. 3 appeared in both runs and thus received both diets. In comparison to the other animals, it excreted a considerably lower volume of urine during the entire test period and therefore, had a higher concentration of allantoin in the urine. The calculated MN supply of each individual was in the range reported by Chen et al. (1992) and Gomes et al. (1994). The animals that received the ensiled partial crop peas had higher mean MN and MNE values than those animals that received native partial crop peas. During ensiling, feed protein is partly hydrolyzed into soluble NPN (Mustafa et al., 2002). Moreover, Ferraretto et al. (2014) and Peyrat et al. (2014) showed in corn and corn silage that increasing protein solubility increased starch accessibility, because the protein matrix which surrounds starch granules was degraded. As a result, starch and oligosaccharides could be more easily fermented by microorganisms and the proportion of fermentable sugars could be increased (Gefrom et al., 2013). Then, energy sources for microbial activity in the rumen are more available in pea silages than in the native material. However, due to the high variation among the animals, we cannot clearly interpret the effect of ensiling on MNE

5. Conclusions

Ensiling of partial crop field peas resulted in a well-preserved protein and fiber rich feed for ruminants. Direct cutting at a late stage of plant maturity (BBCH 81–85) replaced wilting. However, proteolysis was not reduced. The digestibility of CF and detergent fibers decreased in ensiled peas, but OM digestibility and ME and NEL contents remained on a high level. Microbial nitrogen efficiency calculated on the basis of the purine derivative allantoin excreted via the urine was very specific to individual animals, but tendentially increased in sheep that received ensiled partial crop field peas. Ensiling of partial crop field peas maintained the nutritive value and enabled the production of roughage from a local protein source enriched in energy and nutrients.

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Table 4
Concentrations of amino acids in native and ensiled partial crop field peas.

Treatment	Cys	Met	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Tyr	Phe	His	Lys	Arg	Trp
Native	1.28	1.07	11.15	4.18	4.99	15.39	4.31	4.86	4.89	4.91	4.40	7.36	3.22	4.80	2.25	6.71	7.54	1.13
Ensiled	0.97	0.95	10.94	3.75	4.33	14.99	4.01	4.59	4.66	4.78	4.38	6.89	2.67	4.56	2.15	6.30	4.93	0.86

Ala, alanine; Arg, arginine; Asp, aspartic acid; Cys, cysteine; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine.
Amino acids are given in g/16 g nitrogen.

Table 5
Curve fitting parameters for gas production kinetics from the incubation of native and ensiled partial crop field peas modelled with the Gompertz function.

Treatment	*a*	*b*	*c*	*b* + *c*	RMSE	R^2
Native	121[b]	6.3[b]	3.9[b]	10.3	3.10	0.994
Ensiled	156[a]	7.2[a]	4.6[a]	11.7	3.01	0.997

a, asymptotic maximal gas production; *b*, time until which one third of *a* is produced; *b* + *c*, time until which 70% of *a* is produced; *c*, time between *b* and *b* + *c*; RMSE, root mean square error.
a is given in mL/g dry matter, *b* and *c* are given in h.
[ab] Different superscripts indicate differences between the treatments ($P < 0.001$).

Table 6
Calculated daily microbial nitrogen (MN) production and microbial nitrogen efficiency (MNE) based on allantoin excretion via the urine and measured contents of GE in the urine of sheep fed native or ensiled partial crop field peas.

Animal	Body weight	Treatment	Allantoin excretion	MN	MNE	GE
1	53	Native	4.3	2.8	3.4	11.5
2	69	Native	3.7	1.1	1.3	11.1
3	68	Native	16.2	14.0	17.0	11.8
Average				5.9	7.2	11.5
4	64	Ensiled	17.1	14.8	17.7	10.7
3	67	Ensiled	12.2	10.4	12.4	12.3
5	74	Ensiled	7.1	5.7	6.9	11.4
Average				10.3	12.3	11.5

GE, gross energy.
Allantoin excretion is given in mmol/d, MN is given in g/d, MNE is given in g MN/kg digestible organic matter intake and GE is given in MJ/kg dry matter.
Standard deviations were 5.7 and 3.69 g/d (MN), 7.0 and 4.44 g MN/kg digestible organic matter intake (MNE) and 0.330 and 0.630 MJ/kg dry matter (GE) for native and ensiled peas, respectively.

CRediT authorship contribution statement

P. Okon: Investigation, Writing – original draft. **M. Bachmann:** Conceptualization, Methodology, Investigation, Formal analysis, Writing – review & editing. **M. Wensch-Dorendorf:** Statistical analysis, Writing – review & editing. **C. Kuhnitzsch:** Conceptualization, Methodology, Investigation, Writing – review & editing. **S.D. Martens:** Conceptualization, Methodology, Investigation, Writing – review & editing. **J.M. Greef:** Resources, Writing – review & editing. **O. Steinhöfel:** Conceptualization, Resources, Project administration, Funding acquisition, Writing – review & editing. **B. Kuhla:** Resources, Formal analysis, Writing – review & editing. **A. Zeyner:** Conceptualization, Resources, Supervision, Project administration, Funding acquisition, Writing – review & editing. P. Okon and M. Bachmann contributed equally to this work. All authors approved the submitted version of the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 7
Total tract digestibility coefficients of crude nutrients, detergent fibers and energy, and calculated contents of metabolizable energy (ME) and net energy lactation (NEL) in native and ensiled partial crop field peas.

Treatment	OM	CP	AEE	CF	aNDFom	ADFom	ADL	GE	NFE	Starch	ME	NEL
Native	0.85[a]	0.84[a]	0.55[a]	0.77[a]	0.76[a]	0.77[a]	0.32[a]	0.84[a]	0.90[a]	0.97[a]	11.6[b]	7.3[b]
Ensiled	0.85[a]	0.87[a]	0.65[a]	0.68[b]	0.70[b]	0.72[b]	0.35[a]	0.84[a]	0.91[a]	0.97[a]	12.1[a]	7.6[a]

ADFom, acid detergent fiber expressed exclusive of residual ash; ADL, acid detergent lignin; AEE, acid ether extract; aNDFom, neutral detergent fiber treated with amylase and expressed exclusive of residual ash; CA, crude ash; CF, crude fiber; CP, crude protein; GE, gross energy; NFE, nitrogen-free extract; OM, organic matter.
ME and NEL are given in MJ/kg dry matter.
Standard deviations ranged between 0.0050 and 0.125 in native and 0.0048 and 0.13 in ensiled peas, respectively.
[ab] Different superscripts indicate differences between the treatments ($P < 0.05$).

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