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Nutrient composition, fermentation characteristics and mass balance of press juice and press cake obtained from biorefining of grass-clover and red clover silage

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Abstract

Biorefining is seen as a potential method to produce protein-rich feed for monogastric farm animals from grassland, which does not compete with human nutrition. Therefore, a new biorefinery facility was constructed and tested in this experiment by using silages from grass clover mixture and red clover. After biorefining, press juice was stored for further use while press cake was re-ensiled. Samples from silage, press juice, fresh press cake and re-ensiled press cake were analysed for nutrient composition, fermentation parameters, amino acids (AA) and biogenic amines (BA) concentrations. Furthermore, digestibility of silage and re-ensiled press cake was tested in wethers. We found that press juice contained about 205 g crude protein (CP), more than 180 g crude ash and more than 130 g volatile organic compounds (VOC, all values per kg dry matter [DM]). Furthermore, press juice had an unfavourable AA ratio for use as a monogastric feed (methionine and cysteine were reduced). Forage type hardly affected nutritive value of press juice. Re-ensiling of press cake was successful as a sufficient decrease of pH and VOC concentration was observed. Press cake had 26–36 g/kg DM lower CP content and 0.77–1.12 MJ/kg DM lower metabolisable energy content than silage with greater differences in red clover than in grass clover mixture. Press juice can be used as feed in monogastric animals, but its use is limited due to its low CP content and unfavourable AA profile. Press cake could be an appropriate feed for ruminants, especially dry cows or heifers.

KEYWORDS

amino acids, biogenic amines, biorefining, digestibility, energy concentration, re-ensiling

1 | INTRODUCTION

The fractionation of green biomass to produce potential food and feed products is attracting considerable interest. Kromus et al. (2004), Mandl (2010) and Gaffey et al. (2023) define green biorefining as the sustainable processing of biomass into a spectrum of marketable products (protein, minerals, organic acids, energy etc.). The need for more sustainable feed production and the importance of regional protein supply are receiving increasing attention (European Parliament, 2011a). For example, in Austria, a total of 82% of the protein demand for feed can currently be met from self-supply (BMLRT, 2021), with the predominant import demand almost exclusively related to the pig and poultry sectors. Therefore, production of protein-rich feeds for pigs and poultry from green biomass could be an option to reduce the dependency on imports and promote protein security. With a share of more than 50% of agricultural land, grassland

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represents a large potential for green biorefinery in Austria, especially in regions with low livestock numbers and a proportion of grassland worth preserving. According to own evaluations (Resch, 2021), the share of grass silage in the total production of conserved forage was 56% in 2020 with huge differences in forage quality, particularly due to the species group composition (grasses, legumes and herbs) and the time of harvest. Mean crude protein (CP) contents of Austrian silages from permanent grassland, grass/legume mixtures or legumes ranged from 145 to 167 g/kg DM in 2020 (Resch, 2021).

It is estimated that available biorefining technology could convert up to 45% of forage biomass protein into a protein concentrate (Hermansen et al., 2017). However, the method of pressing and the type and configuration of the mechanical press used significantly affects the protein yield (Rinne et al., 2018). According to Franco et al. (2019), silage quality (especially dry matter [DM] and neutral detergent fibre [NDF] content) also significantly influences juice yield and quality and thus refining efficiency. Stødkilde et al. (2021) found that nitrogen (N) content in liquid and solid fractions was significantly affected by crop type (higher when legumes were used as forage crop), growth stage (higher in crops harvested at early vegetation stage) and DM content (higher at lower DM content). Furthermore, the distribution of N between press juice and press cake is highly dependent on the composition of the plant material (Pirie, 1987) and the process parameters used (Colas et al., 2013).

Both fresh grass and grass silage can be used in biorefining. The advantage of grass silage compared to fresh substrates is that it can be processed in green biorefining all year round (Mandl, 2010). The use of press juice from silage in pig diets has been examined by Keto et al. (2021) and Presto Åkerfeldt et al. (2022) who found no adverse effects when including silage press juice into liquid diets of fattening pigs. Furthermore, the press cake could be used in ruminant feeding. Damborg et al. (2019) reported similar milk production responses of dairy cows fed press cake from a grass-clover mixture compared to intact silage harvested 1 week later. Cattle seemed to be able to compensate for the reduced feed quality by increasing forage intake at a low level of press cake inclusion, but higher press cake inclusion resulted in similar feed intake as with intact silage combined with reduced milk output. Mechanical treatment during the pressing process may enhance ruminal degradation and feed intake of cows fed up to 25% of press cake (Savonen et al., 2020).

Previous studies mentioned above show the potential of biorefining of grass products to improve the national availability of protein concentrates in pig and poultry feeding, especially in countries with a high proportion of grassland, like Austria. Therefore, a study on biorefining of grass clover and red clover silage was planned and the following aims were defined: The first aim of this study was to evaluate a new prototype of farm-scale biorefinery facility with regard to feed value, fermentation parameters and mass flow of products (press juice and press cake) and to get information on the nutritional characteristics of press juice for feeding of monogastric animals and of press cake for ruminant feeding. Secondly, the study aimed to examine whether it was possible to re-ensile the press cake without treatment by silage additives and without negative impacts on conservation quality. The third aim was to test if the type of forage crop affects the above mentioned parameters in press juice, fresh press cake and re-ensiled press cake.

2 | MATERIALS AND METHODS

2.1 | Production of silages

In summer 2020, a grass-clover mixture (GCM) and pure red clover (*Trifolium pratense* L.) (RC) were seeded side by side on two neighbouring fields at a satellite station of AREC Raumberg-Gumpenstein (Lambach, Upper Austria, 48°6′ N, 13°53′ E). The two forage crops were seeded side by side as a split plot design would have been too complex for this type of trial. Therefore, it has to be considered that differences between crops could be confounded by a field effect.

The organically operated farm is located 372 m above sea level, with an average annual precipitation of 844 mm. No fertilizers or pesticides were applied on fields until harvest, which took place on 30 May 2021. Two days before harvest, an evaluation of botanical composition was carried out, which resulted in following composition of plant species (values in % of ground covering): 71.7% *Trifolium pratense* L., 6.1% *Trifolium repens* L., 8.8% *Phleum pratense* L., 8.6% *Lolium perenne* L, 3.4% *Dactylis glomerata* L, 1.3% *Festuca pratensis* L; RC: 100.0% *T. pratense*. In both forage crops, red clover was at the phenological stage of inflorescence emergence.

At harvest, both crops were mowed at 12:00 AM with a front-and-rear mower combination (Novacat 265 H, Pöttinger Landtechnik GmbH, Grieskirchen, Austria) equipped with a mower conditioner (ED, Pöttinger Landtechnik GmbH, Grieskirchen, Austria). The cut forage was tedded once immediately after mowing (GF 5001 MH, Kuhn S.A., Saverne, France) and windrowed 24 h after mowing (Eurotop 620A, Pöttinger Landtechnik GmbH, Grieskirchen, Austria). Afterwards, bales were produced using a round baler with a chopping knife (John Deere V451R, Deere & Company, Moline, Illinois, USA) and a theoretical cut length of 11 cm. No silage additives were used and bales were wrapped with eight layers of stretch film. Immediately after harvest, bales were transported to AREC Raumberg-Gumpenstein (Irdning-Donnersbachtal, Styria, Austria, 47°30′ N, 14°6′ E) for storage and further processing.

2.2 | Biorefinery campaign

The bales were used for the biorefinery campaign, which lasted from 6 July, 2021 to 20 July, 2021. At the beginning of the pressing process, stretch film was removed and bales were loaded into a feed mixer (e-stat 6.5 m^3, Scherfler Landtechnik GmbH, Lohnsburg, Austria) by a telehoist load lugger. In the feed mixer, the forage was weighed with an integrated scale and cut to ~5 cm theoretical cut length. Furthermore, a feed sample was taken from the bale before loading into the feed mixer and DM content of this sample was analysed with a microwave oven as described by Losand and Waldmann

(2003). Based on weight and DM content of the bale, water was added to achieve 23% DM content in substrate before pressing. The amount of water added to the forage was recorded for each single bale. After cutting and mixing, the forage was transferred into a mobile mechanical fractionation unit consisting of a (i) feedstock dosing system, (ii) a screw press for solid–liquid separation and (iii) exit lines for solids and liquids produced during biorefining.

The feedstock dosing system consisted of a feedstock storage bunker (type Sieplo 11 m^3, Lunteren, Netherlands) with an integrated scale, which was specifically fitted to dose silage steadily onto a conveyor belt. This conveyor belt transported the feedstock to the dewatering process. For the solid–liquid separation, a small size screw press (type Bellmer-Kufferath Akkupress AXE-LL 250, Düren, Germany) was used. This screw press is a single screw type with a slightly conical screw (average diameter: 250 mm) and a dewatering section (press cage) of ~1400 mm in length. The screw press is driven by a 32 kW electric drive controlled by a frequency inverter. The specific design of the screw press allows the adjustment of the position of the press cage in axial direction. By pushing the press cage onto the conical screw, the geometry (width) of the press outlet can be set and controlled. By monitoring the torque on the screw, the process control system consequently generates commands for setting of the outlet geometry. This process control mechanism is more advanced than 'fixed counter pressor systems' and therefore can avoid potential overloading or disruption of the press. For pressing of the silage, the screw press was set to allow (i) a stable and optimized dewatering of silage (target DM content of press cake: 38%) and (ii) an acceptable throughput of ~1000–1200 kg fresh matter/h (45 Hertz on the drive, cage positioning setting 270–350 mm, maximum average torque value 80 Nm).

The press juice was collected in a juice pan underneath the press and was consequently pumped through a curved sieve (bow screen, 0.25 mm spacing, REKO industrial equipment BV, Stellendam, Netherlands) to separate pulp residues from raw juice. Finally, press juice was transferred into 1250 litre stainless steel storage tanks.

The press cake was transported outside the press on a conveyor belt and stored in a container. Within 4 h after pressing, press cake was re-ensiled with a round baler built for producing maize bales (LT Vario Master, Göweil Maschinenbau GmbH, Kirchschlag bei Linz, Austria).

2.3 | Sampling

Four randomly selected bales per forage type were used for exact evaluation of nutrient composition, fermentation parameters, apparent nutrient digestibility, energy concentration and mass balance. These bales were handled like all other bales except for more intense sampling and data recording. Bales were weighed with a pallet lifter with an integrated scale (VHB, Kern & Sohn GmbH, Balingen, Germany) and samples from three different positions of the bales were collected (self-constructed sampling device) immediately after harvest (wilted forage) and just before start of biorefining (silage). The bale weights were recorded before removing the stretch film and weight of stretch film (3 kg) was subtracted in mass balancing. The single samples were mixed into one mixed sample per bale for further analysis. Furthermore, 60–80 kg of silage (exact weight per bale was recorded) was separated from the bales and frozen for later use in a digestibility trial with wethers.

Afterwards, bales were used for biorefining one by one. Significant precautions were taken to prevent losses of feed material along the biorefining process. However, for the screw press, a complete emptying of the inner screw volume was practically not possible in this study as a complete cleaning of the press would have taken a multiple of time compared to the pressing process itself. Consequently, residual material from a former bale remained inside the press and was pushed out when new material from the next bale entered the system. This circumstance is a potential source of error in mass balancing, especially if batch size is rather small like in this study (batch size was one bale in this study). However, this source of error has to be accepted under the described farm-scale setup and has to be considered when interpreting results on mass balance based on input and output streams of the biorefining process. In our study, we presumed that buffering inside the screw press was 'constant' for all bales.

After the pressing process, weight of press juice and press cake was determined using the pallet lifter with integrated scale and samples of both substrates were taken from each single bale. Before drawing samples from press juice, the liquid was stirred to get a homogeneous sample as solid particles sedimented during storage. Samples were put in a cooling room (temperature at ~4–6°C) immediately after sampling.

Furthermore, four plastic barrels (volume: 60 litre) per bale were filled with press cake for assessing the feed value of re-ensiled press cake (average storage density: ~280 kg DM/m^3). Afterwards, the barrels were hermetically sealed and weighed. The filled barrels were stored at ~20°C for ~8 weeks until opening. After opening, barrels were weighed again and samples were taken from each container using a self-constructed sampling device. Afterwards, samples of one bale were mixed to get a collective sample. Finally, 60–80 kg of re-ensiled press cake were frozen and later used in a digestibility trial with wethers.

2.4 | Feed analysis

Samples for analysis of crude nutrients (CP, ether extract [EE], crude fibre [CF] and crude ash [CA]), cell wall components (neutral detergent fibre assayed with heat stable amylase and expressed exclusive of residual ash [NDF], acid detergent fibre expressed exclusive of residual ash [ADF] and acid detergent lignin [ADL]), sugars and minerals (Ca, P, Mg, K, Na, Fe, Mn, Zn and Cu) were oven-dried at 55°C for 48 h and ground to 1 mm particle size. While all mentioned nutrients were examined in wilted forage, silage and fresh and re-ensiled press cakes, press juice was only analysed for CP, CA, sugars and minerals. The concentrations of above mentioned nutrients were analysed at

AREC Raumberg-Gumpenstein using methods described by VDLUFA (2012) (DM – method 3.1; CP – 4.1.2; EE – 5.1.1; CF – 6.1.1; CA – 8.1; NDF – 6.5.1; ADF – 6.5.2; ADL – 6.5.3; sugars – 7.1.1). The concentration of non-fibre carbohydrates (NFC) was calculated according to Sniffen et al. (1992). Contents of minerals were analysed by inductively coupled plasma emission spectroscopy (iCAP 6000 series, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA).

Amino acids (AA) and biogenic amines (BA) were analysed by the Austrian Agency for Health and Food Safety GmbH (Vienna, Austria) using methods published by VDLUFA (2012). For analysis of AA and BA, freeze-dried (wilted forage, silage, re-ensiled press cake) or frozen samples (press juice) were used. As VOC are lost during the drying process, DM concentration and concentration of all described ingredients were corrected according to Weißbach and Strubelt (2008).

Fermentation parameters (pH, lactic acid, acetic acid, propionic acid, butyric acid, ammonia and ethanol) of silage, press juice and fresh and re-ensiled press cake were analysed in fresh and cooled feed material a few days after sampling in the laboratory at AREC Raumberg-Gumpenstein. The pH value was measured with a pH meter (WTW Multi 3620, Xylem Inc., Washington, DC, USA). The ammonia content was determined using a Kjeltec analyser (Tecator Line Sampler 8460, FOSS, Hillerød, Denmark). The VOC (lactic acid, acetic acid, propionic acid, butyric acid and ethanol) were extracted according to VDLUFA (2012) and measured in a gas chromatograph (3900, Varian Inc., Palo Alto, California, USA), subsequently. Butyric acid is expressed as sum of iso- and n-butyric acid.

2.5 | Determination of in vivo digestibility and energy content of press cake for ruminant feeding

Feeds for the digestibility experiment (silage and re-ensiled press cake from GCM and RC) were thawed 3 days before the digestibility trial. Afterwards, feed material from the four bales per feed was mixed and a sample of each feed was taken for analysis of crude nutrients and cell wall components with the same methods as described above.

The apparent digestibility of feeds was determined according to GfE (1991) using 16 wethers (four wethers per feed). The animal experiment was approved by the Austrian Federal Ministry for Education, Science and Research (document number: BMBWF-66.019/0017-V/3b/2019). The average age of the wethers was 3.6 years, and they had an average live weight of 80 kg. Each wether was fed only one of the four feeds for 19 days with a 14-days adaptation period and a 5-days sampling period. Wethers were assigned to one of the four feeds based on their live weight to assure comparable average live weights in all four experimental groups. Wethers were fed 1 kg DM silage or re-ensiled press cake per day, which was supplemented by 20 g mineral supplement and 4 g salt per day (on fresh matter basis). This ration corresponded approximately to the maintenance requirements of the animals. Animals were fed twice a day at 6:00 AM and 4:00 PM (equivalent amounts of feed per meal). The amounts of feed intake and faeces were recorded daily. Feeds and faeces were analysed once during the sampling period using a pooled sample and the same methods as described above. Additionally, the nitrogen (N) content of faeces was determined in fresh material by method 4.1.1 (VDLUFA, 2012) to prevent N losses during the drying process.

Concentration of metabolisable energy (ME) in silage and re-ensiled press cake was calculated based on concentration of nutrients and in vivo digestibility according to GfE (2001): ME [MJ/kg DM] $= 0.0312 \times$ digestible EE $+ 0.0136 \times$ digestible CF $+ 0.0147 \times$ (digestible organic matter – digestible EE – digestible CF) $+ 0.00234 \times$ CP [each g/kg DM]. The concentration of net energy for lactation (NEL) was also calculated according to GfE (2001): NEL $= 0.6 \times (1 + 0.004 \times ((\text{ME/GE} \times 100) - 57)) \times$ ME [each MJ/kg DM], where GE [MJ/kg DM] $= 0.0239 \times$ CP $+ 0.0398 \times$ EE $+ 0.0201 \times$ CF $+ 0.0175 \times (1000 -$ CP $-$ EE $-$ CF $-$ CA) [each g/kg DM].

2.6 | Mass balance

Based on the weights of the examined bales, the area of the experimental field and the total number of bales, it was possible to calculate the DM yield per ha for GCM and RC as well as an average number of bales per ha. The DM yield of silage, press juice and fresh press cake per ha was estimated by multiplying the recorded DM weights of the respective bale with the average number of bales per ha. For re-ensiled press cake, the relative weight difference (in %) between silage at re-ensiling and silage after opening of the barrels was calculated. Afterwards, the resulting factor was multiplied with the yield of fresh press cake per ha to get the DM yield of re-ensiled press cake per ha. The DM yields per ha of each material were then multiplied with the concentration of CP, NDF, CA, VOC and sugars to get the respective nutrient yields per ha.

The mass balance (MB) was calculated as absolute values (kg DM per ha) and relative values (%). The relative MB of silage was calculated as relative yield in comparison to the wilted forage ($\text{MB}_{\text{Silage}}$ [%] $= \text{Yield}_{\text{Silage}}$ [kg DM/ha]/$\text{Yield}_{\text{Wilted forage}}$ [kg DM/ha] $\times$ 100). In contrast, the mass balance of press juice, fresh press cake and re-ensiled press cake was calculated as relative yield in comparison to silage (e.g., $\text{MB}_{\text{Press juice}}$ [%] $= \text{Yield}_{\text{Press juice}}$ [kg DM/ha]/$\text{Yield}_{\text{Silage}}$ [kg DM/ha] $\times$ 100).

2.7 | Statistical analysis

The data were analysed using the statistical program Statistic Analysis Software 9.4 (SAS Institute Inc., Cary, North Carolina, USA). The procedure mixed was used to evaluate the differences between the two forage crops (C, $i =$ GCM, RC) within the respective feed material (wilted forage, silage, press juice, fresh and re-ensiled press cake) using the model $Y_{ij} = C_i + \varepsilon_{ij}$. Additionally, differences in nutrient composition, apparent digestibility and energy content between the substrates (S) silage and re-ensiled press cake produced from the two forage crops (C, $i =$ GCM, RC) were analysed using the model

$Y_{ijk} = C_i + S_j + (C \times S)_{ij} + \varepsilon_{ijk}$. In analysis of apparent digestibility and energy content, animal was considered as observational unit, while bale was the observational unit in all other analyses. Differences were considered to be significant, if the *p*-value was below .05 and a trend towards significance was assumed if the *p*-value was equal or above .05 and below .10.

3 | RESULTS

3.1 | Nutrient composition and mineral content

Results on DM content, nutrient composition and mineral content of wilted forage, silage, press juice, fresh press cake and re-ensiled press cake are presented in Table 1. Wilted RC had tendentially higher CP and significantly higher NFC concentration, but lower DM content and lower concentration of cell wall components than wilted GCM. Furthermore, content of several minerals (P, Ca, Mg, Cu and Zn) was also higher in RC than in GCM.

Higher CP and NFC content and lower DM and NDF concentrations were also found in RC silage than in GCM silage, as well as higher EE, CA and ADL contents. Regarding minerals, RC silage had higher contents of K, Ca, Mg, Cu and Zn as well as lower Mn content than GCM silage. Furthermore, fermentation was more intense in RC silage as VOC contents were higher and sugar concentration was lower than in GCM silage.

The differences between RC and GCM in VOC and sugar content were also present in press juice and fresh press cake. Regarding the concentration of crude nutrients and cell wall components, most differences in silage were also found in fresh press cake. The only difference was that EE content was higher in GCM press cake than in RC press cake, while in silage it was the opposite.

Re-ensiling of press cake had similar effects in both forage types, as differences found in fresh press cake were also present in re-ensiled press cake, except for CA, organic matter (OM), sugar and VOC content. Furthermore, ADL content was higher in re-ensiled press cake from RC than in that from GCM while it did not differ in fresh press cake. Compared to silage, the CP and NFC content was lower in re-ensiled press cake while concentrations of all other nutrients were higher than in silage.

3.2 | Digestibility and energy content of silage and press cake for ruminant feeding

The use of RC as forage crop resulted in higher apparent CP and NFC digestibility compared to GCM (Table 2). In contrast, apparent NDF digestibility was lower in substrates produced from RC compared to those produced from GCM.

The pressing process reduced the apparent digestibility of CP and NFC and tended to decrease apparent ADF digestibility. For instance, the apparent CP digestibility was 16.5 and 16.8%-units lower in re-ensiled press cakes from GCM and RC compared to silages before pressing. This also resulted in decreased apparent DM and OM digestibility in re-ensiled press cake compared to silage.

Forage type did not affect the ME and NEL content of silage and press cake. However, the ME and NEL content was 0.77–1.12 MJ and 0.60–0.86 MJ lower in re-ensiled press cake compared to silage before pressing.

3.3 | Fermentation quality

The contents of all VOC (lactic acid, acetic acid, butyric acid and propionic acid) was higher in RC silage than in GCM silage (Table 3). As a consequence, pH was also lower in RC silage. The same results were found in press juice and in fresh press cake. However, content of propionic acid in press juice and fresh press cake as well as acetic acid content in press juice were not significantly affected by forage type. Furthermore, there was a trend for a higher ammonia content in fresh press cake from RC compared to GCM.

The fermentation parameters of re-ensiled press cake did not differ between forage types, except for a lower acetic acid content and a higher pH in press cake from RC compared to that from GCM. Compared to silage, lactic acid concentration was markedly higher as well as ethanol and ammonia content and pH were lower in re-ensiled press cake.

3.4 | Amino acids

Due to the higher CP content, concentrations of most AA were higher in wilted forage, silage and re-ensiled press cake from RC compared to those from GCM (Table 4). Only cysteine content in wilted forage and silage as well as aspartic acid content in all three materials were not affected by forage type.

In contrast, no significant difference between forage types was determined in the contents of most AA in press juice. Only the content of proline was significantly higher in press juice from RC compared to that from GCM. Furthermore, there was a trend for a higher glutamic acid, isoleucine and leucine concentration in press juice from RC. However, comparably high residual standard deviation in press juice indicates that variation in AA content was much higher in press juice than in wilted forage, silage and re-ensiled press cake. Except for cysteine, methionine, histidine and arginine, a slight accumulation of AA in press juice compared to silage was found, resulting in slightly lower contents in press cake.

3.5 | Biogenic amines

Figure 1 gives an overview of the concentration of BA in silage, press juice and re-ensiled press cake. Low concentrations of BA were found in the silages before pressing. Six of eight silage samples contained up to 8.17 g/kg DM 4-amino-butyric acid and in two samples of RC silage, small amounts of histamine and tyramine were detected. All

TABLE 1 Concentration of dry matter, nutrients and minerals in wilted forage, silage, press juice, fresh press cake and re-ensiled press cake produced from grass clover mixture (GCM) or red clover (RC).

	Wilted forage			Silage			Press juice			Press cake – fresh			Press cake – re-ensiled		
Parameter	GCM	RC	rSD	GCM	RC	rSD	GCM	RC	rSD	GCM	RC	rSD	GCM	RC	rSD
Dry matter, g/kg FM	346[a]	246[b]	30	316[a]	249[b]	13	115	123	8	376[a]	363[b]	7	369	372	7
Crude protein, g/kg DM	142[y]	162[x]	12	146[b]	159[a]	5	204	208	3	126[b]	141[a]	8	116[b]	126[a]	4
Ether extract, g/kg DM	18.4	17.7	0.9	17.5[b]	22.3[a]	0.5	n.a.	n.a.		21.1[a]	18.2[b]	1.0	28.1[x]	27.1[y]	0.6
Crude fibre, g/kg DM	223[a]	194[b]	10	236	230	11	n.a.	n.a.		316[a]	297[b]	8	300[x]	289[y]	8
Crude ash, g/kg DM	104	109	7	107[b]	111[a]	2	184	186	3	76.6[b]	82.7[a]	2.4	81.2	84.4	2.5
Organic matter, g/kg DM	896	891	7	893[a]	889[b]	2	816	814	3	923[a]	917[b]	2	919	916	2
NDF, g/kg DM	361[a]	310[b]	9	390[a]	343[b]	11	n.a.	n.a.		499[a]	447[b]	9	493[a]	440[b]	8
ADF, g/kg DM	270[x]	251[y]	12	295	309	14	n.a.	n.a.		398	403	17	403	412	14
ADL, g/kg DM	33.5	38.7	5.9	32.9[b]	39.3[a]	2.2	n.a.	n.a.		50.7	55.5	4.3	40.5[b]	47.6[a]	1.3
NFC, g/kg DM	374[b]	401[a]	15	340[b]	365[a]	8	n.a.	n.a.		277[b]	311[a]	12	282[b]	323[a]	4
VOC, g/kg DM	n.a.	n.a.		59.1[b]	85.9[a]	5.3	133[b]	192[a]	19	25.9[b]	38.5[a]	2.8	98.0	93.7	5.1
Sugar, g/kg DM	156	150	4	86.7[a]	40.4[b]	11.6	42.5[a]	17.6[b]	8.8	42.4[a]	20.3[b]	7.9	6.50	5.47	1.0
Phosphorus, g/kg DM	2.93[b]	2.98[a]	0.02	3.02	3.03	0.14	6.61[a]	6.35[b]	0.11	1.53	1.63	0.11	1.53	1.51	0.06
Potassium, g/kg DM	29.0	29.5	1.4	29.6[b]	30.8[a]	0.6	53.2	52.9	1.2	17.8	19.0	0.9	17.2[y]	18.1[x]	0.6
Calcium, g/kg DM	11.3[b]	14.3[a]	0.7	12.3[b]	14.5[a]	0.3	16.6[b]	18.3[a]	0.4	10.2[b]	13.0[a]	0.7	10.2[b]	12.3[a]	0.2
Magnesium, g/kg DM	2.24[b]	2.67[a]	0.09	2.31[b]	2.80[a]	0.10	4.37[b]	4.91[a]	0.12	1.47[b]	1.86[a]	0.05	1.52[b]	1.87[a]	0.05
Sodium, mg/kg DM	65.1	76.7	21.2	92.8	104.8	31.3	550	439	123	73.8[b]	94.6[a]	6.6	172	138	45
Iron, mg/kg DM	460	442	211	447	519	55	840	911	138	527	612	64	676	743	93
Copper, mg/kg DM	6.66[b]	8.38[a]	0.44	6.95[b]	8.63[a]	0.29	6.06[b]	7.09[a]	0.30	7.39[b]	9.21[a]	0.49	6.93[b]	8.80[a]	0.35
Zinc, mg/kg DM	26.4[b]	30.8[a]	2.2	25.7[b]	28.1[a]	0.8	70.9	66.7	12.9	20.0[b]	23.2[a]	0.9	19.2[b]	21.0[a]	0.99
Manganese, mg/kg DM	47.2	41.5	10.3	50.8[a]	44.7[b]	3.4	84.9[a]	68.6[b]	4.3	37.8	34.9	2.2	40.7	37.5	3.7

Note: Different superscripts indicate a significant difference (a, b) or a trend for a difference (x, y) between forage crops (GCM, RC).

Abbreviations: ADF, acid detergent fibre expressed exclusive of residual ash; ADL, acid detergent lignin; DM, dry matter; FM, fresh matter; n.a., not analysed; NDF, neutral detergent fibre assayed with heat stable amylase and expressed exclusive of residual ash; NFC, non-fibre carbohydrates; rSD, residual standard deviation; VOC, volatile organic compounds.

TABLE 2 Differences in nutrient composition, in vivo-digestibility and energy concentration between silage and re-ensiled press cake produced from grass clover mixture (GCM) or red clover (RC).

Substrate	Silage		Press cake – re-ensiled			Statistical significance (*p*-value)		
Forage crop	GCM	RC	GCM	RC	rSD	Forage crop	Substrate	Forage crop × substrate
Nutrient composition								
Dry matter, g/kg fresh matter	316[b]	249[c]	370[a]	372[a]	10	<0.001	<0.001	<0.001
Crude protein, g/kg DM	146	159	116	126	4	<0.001	<0.001	0.498
Ether extract, g/kg DM	17.5[c]	22.3[b]	28.1[a]	27.1[a]	0.6	<0.001	<0.001	<0.001
Crude fibre, g/kg DM	236	230	300	289	10	0.084	<0.001	0.576
Organic matter, g/kg DM	893	889	919	916	2	0.007	<0.001	0.681
Neutral detergent fibre, g/kg DM	390	343	493	440	10	<0.001	<0.001	0.609
Acid detergent fibre, g/kg DM	295	309	403	412	14	0.124	<0.001	0.720
Acid detergent lignin, g/kg DM	32.9	39.3	40.5	47.6	1.8	<0.001	<0.001	0.679
Non-fibre carbohydrates, g/kg DM	340[b]	365[a]	282[d]	323[c]	7	<0.001	<0.001	0.044
Digestibility								
Dry matter, %	72.3	74.0	66.3	64.9	2.3	0.926	<0.001	0.234
Crude protein, %	62.8	68.5	46.3	51.7	3.2	0.010	<0.001	0.930
Ether extract, %	49.3	47.4	50.4	47.8	4.3	0.351	0.756	0.878
Crude fibre, %	65.6	62.7	67.1	66.0	2.6	0.182	0.124	0.526
Organic matter, %	74.7	76.6	67.6	66.7	2.1	0.666	<0.001	0.243
Neutral detergent fibre, %	64.6	58.4	63.4	54.8	3.5	0.003	0.221	0.536
Acid detergent fibre, %	63.9	62.4	62.0	56.6	3.4	0.087	0.057	0.314
Non-fibre carbohydrates, %	91.2	94.5	84.3	89.0	1.1	<0.001	<0.001	0.261
Energy content								
Metabolizable energy, MJ/kg DM	10.21	10.46	9.44	9.34	0.27	0.628	<0.001	0.267
Net energy for lactation, MJ/kg DM	6.14	6.33	5.54	5.47	0.19	0.608	<0.001	0.237

Note: Different superscripts indicate a significant substrate × forage crop interaction.
Abbreviations: DM, dry matter; rSD, residual standard deviation.

other silage samples had BA contents lower than the determination level.

In three samples of re-ensiled press cake, cadaverine contents of ~3 g/kg DM were detected. Furthermore, putrescine concentration was between 0.90 and 1.84 g/kg DM (except for one sample with not determinable content) as well as tyramine concentration was between 1.19 and 1.79 g/kg DM in re-ensiled press cakes. Concentration of 4-amino butyric acid was on a similar or even lower level than in silage.

In contrast, comparably high concentrations of BA were found in press juice. Maximum cadaverine, histamine, phenethylamine, putrescine, tyramine and 4-aminobutyric acid concentrations were 14.5, 8.76, 0.16, 7.46, 4.95 and 56.0 g/kg DM. High levels of BA were detected in press juice both from GCM and RC, except for phenethylamine which was present only in press juice from GCM.

3.6 | Mass balance

The NDF and sugar yield per ha (wilted forage and silage) was higher in GCM than in RC while CP and CA yield did not differ between forage types (Table 5). Furthermore, a higher VOC yield was found for RC silage compared to GCM silage. For press juice, the yield of DM and all analysed nutrients was influenced by the forage type. The use of RC lead to higher CP and VOC ($p < .05$) as well as DM and CA ($0.05 \le p < .10$) yield per ha and to lower sugar yield per ha ($p < .05$). In contrast, forage type had only little effect on yield per ha of fresh and re-ensiled press cake. The only difference was a higher VOC yield per ha and a lower sugar yield per ha of fresh press cake when using RC instead of GCM.

Although the pressing of silage resulted in DM being attributed 25.5%–27.7% to the press juice, 67.8 and 68.6% to the press cake and 3.7%–6.7% to process losses, 35.7%–36.4% of CP, 44.0%–46.5% of CA and 57.1%–61.8% of VOC were transferred to press juice, which indicated that the press juice was enriched with CP, CA and VOC. The transfer rates of DM, CP, CA and VOC to press juice and fresh press cake were higher for RC in comparison to GCM. Less than 50% of sugar present in silage was transferred to press juice (12.6% and 12.4% in GCM and RC) and fresh press cake (33.4% and 34.4% in GCM and RC) meaning that more than 50% of sugar got lost during the biorefining process. Compared to fresh press cake, the relative CP and sugar yield (as a % of silage yield) was lower and the

TABLE 3 Concentration of volatile organic acids, ethanol, ammonia as well as pH in silage, press juice, fresh press cake and re-ensiled press cake produced from grass clover mixture (GCM) or red clover (RC).

	Silage			Press juice			Press cake – fresh			Press cake – re-ensiled		
Parameter	GCM	RC	rSD	GCM	RC	rSD	GCM	RC	rSD	GCM	RC	rSD
Lactic acid, g/kg DM	36.5[b]	56.9[a]	5.0	81.3[b]	128.2[a]	14.2	15.4[b]	24.4[a]	2.4	75.3	71.3	5.7
Acetic acid, g/kg DM	11.4[b]	14.2[a]	0.9	26.9	31.2	3.2	5.26[b]	6.53[a]	0.50	14.0[a]	13.4[b]	0.3
Butyric acid, g/kg DM	3.54[b]	5.97[a]	1.01	8.63[b]	14.59[a]	2.47	1.47[b]	2.66[a]	0.37	2.87	3.86	0.77
Propionic acid, g/kg DM	1.55[b]	2.14[a]	0.30	4.48	4.22	0.28	1.10	1.13	0.09	0.945	0.957	0.140
Ethanol, g/kg DM	6.11	6.70	0.86	11.8	13.5	1.6	2.67	3.75	1.05	4.85	4.23	0.79
Ammonia, g/kg DM	2.33	2.68	0.31	5.90	6.35	0.33	1.08[y]	1.24[x]	0.10	1.27	1.24	0.05
pH	4.75[a]	4.58[b]	0.08	4.75[x]	4.63[y]	0.09	4.71[x]	4.59[y]	0.07	4.10[b]	4.22[a]	0.02

Note: Different superscripts indicate a significant difference (a, b) or a trend for a difference (x, y) between forage crops (GCM, RC).
Abbreviations: DM, dry matter; rSD, residual standard deviation.

TABLE 4 Amino acid concentration in wilted forage, silage, press juice and re-ensiled press cake produced from grass clover mixture (GCM) or red clover (RC).

	Wilted forage			Silage			Press juice			Press cake – re-ensiled		
Amino acid	GCM	RC	rSD	GCM	RC	rSD	GCM	RC	rSD	GCM	RC	rSD
Cysteine, g/kg DM	1.05	1.17	0.11	0.664	0.736	0.073	n.d.	n.d.		0.754[b]	0.911[a]	0.052
Methionine, g/kg DM	2.05[b]	2.18[a]	0.07	1.77[b]	1.96[a]	0.09	n.d.	n.d.		1.69[b]	1.90[a]	0.12
Cysteine+Methionine, g/kg DM	3.10[y]	3.35[x]	0.16	2.44[b]	2.70[a]	0.11	2.45	2.87	0.63	2.45[b]	2.81[a]	0.13
Aspartic acid, g/kg DM	18.2	18.7	0.5	16.1	15.7	0.7	24.3	22.0	4.5	11.2	11.7	0.5
Threonine, g/kg DM	5.77[b]	6.39[a]	0.20	5.12[b]	6.00[a]	0.15	7.18	8.20	1.20	4.60[b]	5.31[a]	0.11
Serine, g/kg DM	5.85[b]	6.56[a]	0.22	4.71[b]	5.40[a]	0.17	5.94	6.30	1.08	4.24[b]	5.00[a]	0.19
Glutamic acid, g/kg DM	13.6[b]	14.9[a]	0.4	10.8[b]	12.7[a]	0.2	13.4[y]	16.0[x]	1.9	9.08[b]	10.49[a]	0.37
Proline, g/kg DM	7.96[b]	9.97[a]	0.38	7.23[b]	9.11[a]	0.32	9.86[b]	13.74[a]	1.87	5.49[b]	6.43[a]	0.35
Glycine, g/kg DM	6.49[b]	7.20[a]	0.20	5.87[b]	6.68[a]	0.17	6.97	8.02	1.12	5.46[b]	6.12[a]	0.22
Alanine, g/kg DM	7.71[b]	8.29[a]	0.29	7.89[b]	8.94[a]	0.20	10.7	12.2	1.5	6.53[b]	7.13[a]	0.23
Valine, g/kg DM	7.07[b]	8.10[a]	0.27	7.45[b]	8.51[a]	0.24	8.82	10.51	1.30	6.12[b]	6.79[a]	0.26
Isoleucine, g/kg DM	5.71[b]	6.56[a]	0.27	5.95[b]	6.95[a]	0.23	6.97[y]	8.59[x]	1.11	5.05[b]	5.62[a]	0.23
Leucine, g/kg DM	10.2[b]	11.5[a]	0.4	9.94[b]	11.53[a]	0.28	11.1[y]	13.5[x]	1.7	8.58[b]	9.60[a]	0.29
Tyrosine, g/kg DM	4.05[b]	4.61[a]	0.17	3.43[b]	4.28[a]	0.13	3.81	4.96	0.79	2.78[b]	3.31[a]	0.17
Phenylalanine, g/kg DM	6.32[b]	7.06[a]	0.23	5.84[b]	6.60[a]	0.15	7.20	8.02	0.80	5.59[b]	6.25[a]	0.23
Histidine, g/kg DM	2.55[b]	2.93[a]	0.08	1.88[b]	2.43[a]	0.10	1.20	1.73	0.78	1.95[b]	2.45[a]	0.13
Lysine, g/kg DM	6.49[b]	7.20[a]	0.23	5.04[b]	5.53[a]	0.22	7.14	6.68	1.66	4.47[b]	5.88[a]	0.32
Arginine, g/kg DM	6.04[b]	6.65[a]	0.28	3.88	3.90	0.30	3.26	2.48	0.86	3.49[b]	3.93[a]	0.14

Abbreviations: DM, dry matter; n.d., not determinable; rSD, residual standard deviation.
Note: Different superscripts indicate a significant difference (a, b) or a trend for a difference (x, y) between forage crops (GCM, RC).

relative VOC yield was higher in re-ensiled press cake, while relative DM yield of fresh and re-ensiled press cake was comparable.

4 | DISCUSSION

This study aimed to evaluate the feed value and fermentation quality of press juice (for feeding of monogastric farm animals) and press cake (for feeding of ruminants) produced by a farm-scale green biorefinery. Furthermore, the possibility of re-ensiling of press cake was tested and effects of two forage types (GCM and RC) on characteristics of press juice and press cake were analysed. To produce high quality press juice and press cake, good quality silage is required. The nutrient composition, VOC content, apparent OM digestibility and energy content of the silages used in this study were within the range of thousands of grass silage samples analysed in Austrian forage projects in

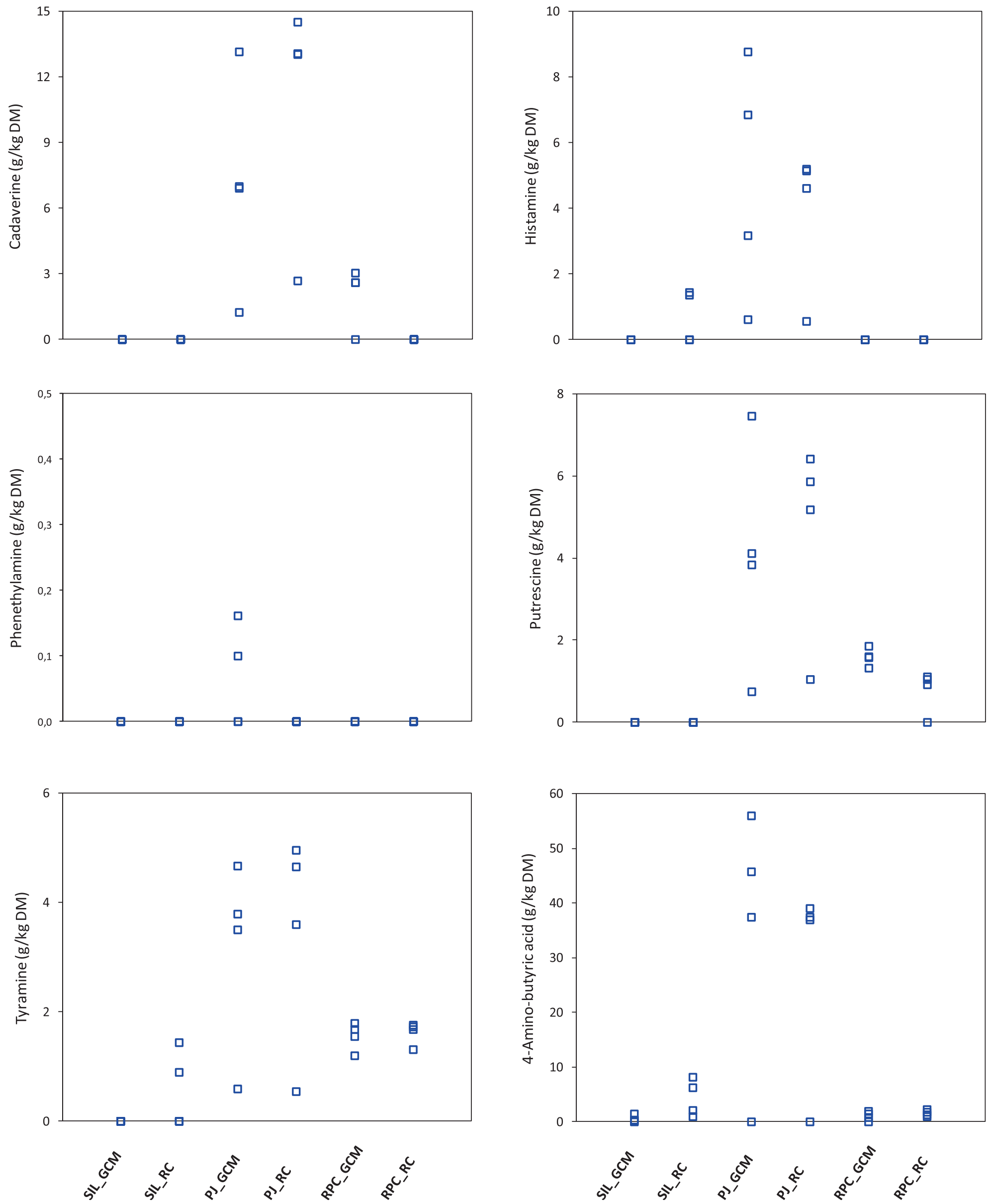

FIGURE 1 Concentration of the biogenic amines cadaverine, histamine, phenethylamine, putrescine, tyramine and 4-amino butyric acid in silage (SIL), press juice (PJ) and re-ensiled press cake (RPC) produced from grass-clover mixture (GCM) or red clover (RC) ($n = 4$ samples per feed). Samples with concentration lower than determination level are presented as 0. If less than four squares are visible per feed, more than one sample had a concentration below the determination level.

the last 20 years (Resch, 2021). Additionally, it can be assumed that nutritive value und fermentation quality of silages used in this biorefining project was better than the average in the studies by Resch (2021) as CF, NDF and butyric acid contents were lower than the average.

4.1 | Nutritive value and fermentation parameters of press juice

One aim of the green biorefining process was to produce a protein-rich press juice with a favourable AA profile as a monogastric animal feed. With CP contents of 204–208 g/kg DM, sugar contents of 17.6–42.5 g/kg DM and negligible fibre fractions, press juice from GCM and RC is likely to be classified as a protein feed. Although press juice CP content was similar compared to studies conducted by Franco et al. (2018), Presto Åkerfeldt et al. (2022) and Keto et al. (2021) (157–290 g/kg DM), when compared to commonly used protein feeds, major differences were apparent: the CP content of GCM and RC press juice was less than half that of soybean meal and soybean cake (500 and 455 g/kg DM) and less than a third of maize gluten and potato protein concentrates (670 and 840 g/kg DM). However, it was only slightly lower than in the grain legumes peas and fava beans (250 and 300 g/kg DM, DLG (2014)). Additionally, energy-rich residual oil and starch, as found in the by-products of soya oil production and in grain legumes, are expected to being low in press juice due to the low EE and starch contents in grass products and the pressing process, again underlining that it can be considered a protein feed. Content of CA, on the other hand, was three times higher in the press juice than in soybean meal and soybean cake. As a consequence of its production process, the silage press juice had a very low DM content. Therefore, it will be necessary to either keep the feed intake

TABLE 5 Mass balance of the biorefinery campaign using grass-clover mixture (GCM) or red clover (RC) as forage crop.

		Wilted forage			Silage[1]			Press juice[2]			Press cake – fresh[2]			Press cake – re-ensiled[2]		
Parameter		GCM	RC	rSD	GCM	RC	rSD	GCM	RC	rSD	GCM	RC	rSD	GCM	RC	rSD
Dry matter	kg/ha	3033	2795		2755	2778	202	701^{y}	769^{x}	45	1868	1909	269	1832	1950	286
	%				90.8	99.4		25.5	27.7		67.8	68.6		66.6	70.1	
Crude protein	kg/ha	432	454	36	402	442	37	143^{b}	160^{a}	9	235	267	31	213	246	37
	%				93.9	97.7		35.7	36.4		58.6	60.7		53.1	55.6	
Crude ash	kg/ha	315	305	20	294	307	23	129^{y}	143^{x}	8.3	144	158	24	149	165	26
	%				93.6	101.7		44.0	46.5		48.9	51.4		51.0	53.5	
NDF	kg/ha	1096^{a}	867^{b}	28	1074^{a}	951^{b}	65	n.a.	n.a.		930	853	116	900	858	126
	%				97.9	109.7					86.7	89.8		84.0	90.3	
VOC	kg/ha	n.a.	n.a.		163^{b}	238^{a}	19	93.0^{b}	147.5^{a}	14.6	48.4^{b}	73.9^{a}	12.2	180	183	33
	%							57.1	61.8		29.9	31.1		113.2	76.9	
Sugar	kg/ha	473^{a}	420^{b}	13	240^{a}	112^{b}	41	29.6^{a}	13.6^{b}	6.0	81.2^{a}	39.2^{b}	22.5	12.1	10.6	3.0
	%				50.7	26.8		12.6	12.4		33.4	34.4		5.0	9.5	

Note: Different superscripts indicate a significant difference (a, b) or a trend for a difference (x, y) between forage crops (GCM, RC).

Abbreviations: n.a., not analysed; NDF, neutral detergent fibre assayed with heat stable amylase and expressed exclusive of residual ash; VOC, volatile organic compounds.

[1]Relative values in % of wilted forage (not statistically analysed).

[2]Relative values in % of silage (not statistically analysed).

capacity of the receiving monogastric animals in mind when it is fed fresh, or to explore drying option.

Protein extraction rate from silage to press juice was 36% in this trial. This protein extraction rate is comparable to the results achieved by Colas et al. (2013) who used alfalfa, which was frozen and thawed prior to biorefining. However, according to Ayanfe et al. (2023), ensiling of grasses increases protein extraction rate compared to fresh grass, dried grass or grass which was frozen and thawed before processing. However, higher protein degradation is a disadvantage of silage compared to fresh feed material (Rinne, 2024). In earlier experiments, markedly higher protein extraction rate (50% in Colas et al. (2013), 41% and 53% in Rinne et al. (2018)) or nitrogen extraction rate (47% and 65% Stødkilde et al. (2021)) was achieved by using double srew pressing methods. Therefore, protein extraction rate was on a comparably low level in our study and could be increased by using different types of presses in the biorefinery process. This would likely be also favourable with regard to the protein content of the press juice.

Besides CP content, AA profile is another important feed value parameter in feeding of monogastric animals. The first-limiting and therefore most relevant AA in pig feeding is lysine, while in poultry feeding the sulphur-containing AA methionine and cysteine are first-limiting. The lysine content in GCM and RC press juice (7.1 and 6.7 g/kg DM) was lower than the German recommendation for the total diet of fattening pigs weighing 60–90 kg (9.7 g/kg DM, Kirchgessner et al. (2011)). The methionine+cysteine content in the press juice (2.5 and 2.9 g/kg DM) was less than half of the recommendation for the total diet of laying hens (5.5–6.0 g/kg DM, GfE (1999)). Soybean meal and soybean cake, on the other hand, are much higher in lysine (31.0 and 27.0 g/kg DM), and the protein concentrates maize gluten and potato protein are much higher in methionine+cysteine (28.4 and 31.5 g/kg DM, DLG (2014)). Previous reports about silage press juice found both similar (Presto Åkerfeldt et al. (2022), 6.9 g/kg DM) and higher lysine contents (Keto et al. (2021), 13.6 g/kg DM), most likely caused by differences in the botanical composition of the substrate and technical aspects of the production process. A closer look at the AA profile of the tested GCM and RC press juice shows that lysine made up 3.2% and 3.5% of the total CP, which is about half of the concentration in soybean meal and soybean cake (5.9 and 6.2%), peas and fava beans (6.1 and 7.2%, DLG (2014)). Methionine+cysteine made up 1.2 and 1.4% of total CP in the tested press juice, which was less than half of the concentration in peas and fava beans (2.9 and 2.1%), and less than a third compared to potato protein and maize gluten (3.8 and 4.2%, DLG (2014)). For the growing pig, the optimal relationship between the most relevant essential AA lysine: (methionine +cysteine): threonine is 1: 0.52–0.60: 0.62–0.66 (GfE, 2006), while for laying hens the ideal ratio is 1: 0.87: 0.71 (GfE, 1999). The GCM and RC press juice had a ratio of 1: 0.34–0.43: 1.01–1.23, showing a relatively low concentration of methionine+cysteine, and a high concentration of threonine. While the ratio of methionine+cysteine to lysine was lower than recommended both for fattening pigs and laying hens, it is especially relevant for hen and poultry in general. To summarize, both the total contents of the first-limiting AA as well as the AA profile of GCM and RC press juice was found to be unfavourable compared to other commonly used protein feeds. It is therefore clear, that while GCM and RC press juice can supply valuable AA to monogastric farm animals, other components higher in the first-limiting AA will be needed to balance diets. Therefore, press juice can replace other protein-rich feeds only to certain extent. The maximum possible press juice proportion in pig's or poultry's diets should be examined in feeding experiments.

The forage type did not significantly affect the content of AA in press juice in our experiment and in a study by Rinne (2024) who found only minor differences in AA profiles of press juices produced from red clover or timothy (*Phleum pratense*). However, management factors during ensiling may also influence the AA profile of silage and therefore of press juice. Unfavourable conditions during ensiling result in higher proteolysis and worsen the AA profile (Rinne, 2024) while addition of formic acid (Rinne, 2024) and high DM content of silage (Tian et al., 2023) reduce proteolysis and promote conservation of a favourable AA profile.

Ammonia and biogenic amines are products of proteolytic processes and both were enriched in press juice compared to silage in the current study as these substances are part of the soluble protein. Ammonia is a toxic gas and considered a main health hazard for pigs in closed barns. However, as part of the diet it might also supply nitrogen, as Mansilla et al. (2017) reported that pigs can use nitrogen from ammonia as efficiently as from protein when diets are deficient in non-essential amino acids. Therefore, it remains unclear if the ammonia content of the press juice could limit the inclusion in pig diets. Biogenic amines are also a group of toxic substances which could be harmful to animals if there are high concentrations in a feed. Concentration of BA in silage and re-ensiled press cake were in the range of values reported by Scherer et al. (2015), except for histamine content which was higher in two of four red clover silages used in this experiment. However, BA concentration in press juice was massively higher than in silages in the Scherer et al. (2015) review. According to Rooke and Hatfield (2003), deamination, decarboxylation and oxidation are the processes which lead to proteolysis. According to these authors, clostridia, which are likely to appear in the press juice due to unavoidable soil contamination in silages, degrade lysine to cadaverine by decarboxylation. Furthermore, Li et al. (2022) performed a PCR microbiome analysis and identified *Escherichia coli* and *Klebsiella oxytoca* as further species producing cadaverine and tyramine. One reason for high BA content in this study could be the high acetic acid and ammonia contents in press juice which are positively correlated to concentration of BA according to van Os et al. (1996). Another possible reason for high BA concentration in press juice could be the high temperatures (>30°C in afternoon) during the biorefining trial which was carried out in July 2021. Although samples were cooled immediately after drawing, these high temperatures could have promoted deterioration processes in press juice. Regarding the effect of high BA concentration in feeds on feed intake of animals, results of previous studies are contradictory. While Scherer et al. (2015) did not find marked effects of BA in silages on feed intake of ruminants, DM intake of steers was reduced by high concentrations of cadaverine and putrescine in alfalfa silage in a study by Phuntsok et al. (1998).

Ammonia and BA contents in press juices from biorefining and factors influencing their contents should be examined more in detail in future studies. Furthermore, research on effects of BA on yields and health of monogastric animals are of interest as the authors are not aware of any study that has already examined this. Based on the current knowledge, it is still unclear how ammonia and BA concentrations limit the use of press juice in animal feeding. However to be safe, the ammonia and BA concentrations should be at least considered in the formulation of rations containing press juice.

Another result of this study was that CA content of the tested GCM and RC press juice was much higher than in the original silage, and also higher than in previous reports by Presto Åkerfeldt et al. (2022) (120.4 g/kg DM). Looking at the macro minerals, the calcium content in the press juice was almost three times higher than the German recommendation (Kirchgessner et al., 2011) for the total diet of fattening pigs. Furthermore, phosphorus and potassium contents in press juice were also higher than recommended for pigs (7.8–10.0 g potassium/kg feed [NRC, 2012]; 4.5 g total phosphorus/kg DM [Kirchgessner et al., 2011]). In contrast, phosphorus content of press juice met the phosphorus requirement of laying hens (6.5 g total phosphorus/kg DM [Jeroch et al., 2012]), while calcium content was only half of that recommended for lying hens diets (39.2 g/kg DM [GfE, 1999]). The iron content was on a similar level like in the study by Keto et al. (2021; 700 mg iron/kg DM of silage press juice) and about 15 times the German recommendation for fattening pigs (50–60 mg/kg DM, Kirchgessner et al. [2011]), and more than eight times the recommendation for laying hens (100 mg/kg DM, GfE (1999)). No reports of iron toxicity in fattening pigs are known to the authors, and increased intake should merely result in increased excretion. However, there are legal upper limits for iron supply to pigs (GfE, 2006), as well as for poultry, which are exceeded by the press juice (750 mg iron/kg total diet (Jeroch et al., 2012)). Therefore, high iron content of press juice should be considered in the creation of mixed feeds for monogastric animals. Assuming an inclusion rate of 15% in diets for fattening pigs (which is common for diets in organic pig fattening), the contents of macro minerals will not limit the inclusion of press juice in pig diets. Depending on the remaining feed components, iron concentration will most likely not exceed the legal upper limit, but will be higher than the feeding recommendations. In diets for laying hens, neither the macro nor micro minerals in press juice are likely to limit the inclusion of press juice, both because egg production comes with an increased demand for minerals, and because poultry diets typically combine several protein feeds. However, both for pigs and poultry, it is advisable to consider the mineral concentrations in diet formulation to reduce the need and cost of mineral feed.

Because the press juice was produced from silage, a relevant concentration of volatile organic acids was found in it. In organic pig feeding, the use of silage is widespread because of the mandatory provision of roughage as a manipulable material, and also because it supplies nutrients (Wüstholz et al., 2017) and has a positive impact on gastrointestinal health. One of the main reasons for this positive effect is lactic acid, which has been found to act as a probiotic in pig diets (Yang et al., 2015). Positive effects of including organic acids in the diet on gastrointestinal health and growth have also been found by Partanen and Mroz (1999) and Suiryanrayna and Ramana (2015). The contents of lactic and acetic acid found in the GCM and RC press juice were similar and slightly lower, respectively, than those reported by Keto et al. (2021) for press juice that was successfully included in liquid feeding of fattening pigs. In poultry, the provision of silage is also mostly relevant for organic farms, but because of restrictions in feed intake capacity, the supply of nutrients from silage is limited. However, as for pigs, there are reports about a positive effect of lactic acid on health in broilers (Valečková et al., 2020), gastrointestinal health in laying hens (Steenfeldt et al., 2007) and an antimicrobial effect of organic acids in general (Hajati, 2018). When including a mixture of lactic, butyric, propionic and formic acid in diets for laying hens, Soltan (2008) even found positive effects on egg production. Based on these reports, it can be assumed that the contents of organic acids in the tested press juice would not impair animal performance when fed to pigs and poultry. Ammonia, which was also present in the tested press juice, is a toxic gas and considered a main health hazard for pigs in closed barns. However, as part of the diet it might also supply nitrogen, as Mansilla et al. (2017) reported that pigs can use nitrogen from ammonia as efficiently as from protein when diets are deficient in non-essential amino acids. Therefore, it remains unclear if the ammonia content of the press juice could limit the inclusion in pig diets.

Although press juice from grass silage has disadvantages compared to other protein-rich feeds regarding nutritive value and especially regarding the AA profile, it is worthwhile to put more effort in improving the nutritive value of such press juices. Animal feeds produced from grassland do not compete with human nutrition. Although by-products from food production are often considered as products which are not appropriate for human nutrition, Ertl et al. (2016) stated that 50% of protein in soybean cake and 30% of protein in rapeseed cake is human-edible. If everything is optimized and extraction of protein is maximized, 92% of protein in soybean cake and 87% of protein in rapeseed cake could be used for human nutrition according to their assumptions. Therefore, using protein concentrates from grassland could markedly reduce the use of human-edible feed in pig and poultry production. Although the press juice could also be used as human food if high quality is ensured, the regulations regarding food safety and food declaration make it very difficult to produce food from grassland. Especially regulations regarding the exact listing of ingredients (European Parliament, 2011b) are hard to fulfil as grassland is a heterogeneous mixture of different species.

4.2 | Fermentation parameters and nutritive value of re-ensiled press cake

Re-ensiling of press cake is a measure which allows storing of press cake and a more flexible use in ruminant feeding. Although the sugar content of fresh press cake was only half of that in silage, the fermentation quality of re-ensiled press cake was good or even better than in silage (e.g., higher lactic acid and lower butyric acid and ethanol content). This is in accordance with results from Larsen et al. (2019), who were successful in producing silage from press cake of fresh GCM and ryegrass. According to those authors, a short period between processing and ensiling is crucial to achieve a good acidification of the silage. Furthermore, Damborg et al. (2019) also found higher lactic acid content as well as lower pH in ensiled press cake produced from fresh GCM compared to GCM silage. These authors related the higher lactic acid production in press cake to a lower DM content compared to the GCM silage which was produced independently from the GCM used for biorefining. The good fermentation characteristics of re-ensiled press cake were unexpected in this study as sugar content of fresh press cake was low (42.4 and 20.3 g/kg DM in fresh press cake from GCM and RC). However, the good conservation success could be explained by biochemical hydrolysis of polysaccharides from the NFC pool or even from cell wall with increasing fermentation period of grassland feeds. This hydrolysis results in short-chain carbohydrates which can be metabolized by lactic acid bacteria during the process of re-ensiling (Rooke & Hatfield, 2003). Furthermore, a lower buffering capacity due to lower CP and minerals content in fresh press cake compared to wilted forage could have contributed to the favourable fermentation characteristics of press cake. In summary, the good fermentation quality of re-ensiled press cake is an important prerequisite for all-year use of press cake in ruminant feeding.

The VOC and sugar content of re-ensiled press cake did not differ between forage types although silage and fresh press cake from RC had higher VOC (especially higher content of lactic and butyric acid) and lower sugar concentration than the corresponding products from GCM. The reason for the high VOC content in silage and fresh press cake from RC could be a higher buffer capacity of legumes due to a higher content of protein and minerals (McDonald & Henderson, 1962) as found for pure RC in this study. Therefore, ensiling of legumes requires higher production of acids to decrease the pH below the critical value of 5.0 and to prevent proliferation of clostridia. Protein and mineral contents in fresh press cake of both forage types were on a low level which might explain the lack of differences in VOC concentration in re-ensiled press cake.

The feed value of re-ensiled press cake was comparable with the lower quarter of grass silage (first cut) analysed in the projects by Resch (2021). Especially lower CP and energy content have to be considered in formulation of ruminant rations to achieve comparable yields. The lower energy content of re-ensiled press cake compared to silage was due to the lower apparent digestibility of the press cake. According to Hansen et al. (2023), double screw pressing of grass silage could increase NDF digestibility of press cake which might also increase energy content. However, the effect of increased NDF digestibility was only found when feed harvested in late vegetation stage was used for biorefining, and not when using early harvested feed. Furthermore, it has to be considered that apparent CP digestibility was also quite low in the current study. Due to the low CP digestibility of press cake, feeding of press cake to ruminants requires a higher CP supply to provide animals with adequate amounts of digestible protein.

The nutritive value of re-ensiled press cake was influenced by the forage type used. The re-ensiled press cake from RC had higher CP and NFC as well as lower NDF content compared to re-ensiled press cake from GCM. In the study published by Stødkilde et al. (2021), nitrogen content was also higher in fresh press cake produced from legumes compared to that produced from grasses. The re-ensiled press cake from RC had a lower apparent NDF digestibility compared to press cake from GCM, which is likely due to the higher lignin (ADL) content of these feeds. The content of lignin in a feed, but also the composition of lignin significantly influences the digestibility of feeds (Wilson, 1994). Cellulose and hemicellulose are fully digestible but lignin forms complexes with other cell components making them indigestible (Wilson, 1994). However, the lower apparent NDF digestibility of press cake from RC was compensated by higher apparent NFC digestibility resulting in comparable apparent OM digestibility in both forage types. Based on our results, press cake has to be classified as a forage with low to medium nutritive value, whereat nutritive value of press cake from RC is slightly higher due to a higher CP content.

5 | CONCLUSION

Press juice from GCM and RC produced by a new green biorefinery process contained relevant levels of CP and essential AA, as well as organic acids with potential health benefits. The forage type (grass clover mixture vs. red clover) had a minimal impact on the press juice's nutritive value. Therefore, press juices from both silages can be used as feed for monogastric animals, but there are some limitations which have to be kept in mind. When used in pig and poultry diets, attention should be paid to the high mineral contents, and to balancing the AA profile of the total diet. The palatability of the press juice as well as how it can be incorporated into total diets needs to be assessed. Concentrations of certain BA have to be considered as they were significantly increased in press juice compared to silage. In summary, the comparably low nutritive value and relatively high contents of minerals and BA may limit the proportion of press juice in the ration. Further research should focus on how mineral and BA content can be reduced by adapted technical procedures. Re-ensiling of press cake is an appropriate method for storing as lactic acid concentration was higher and pH value was lower than in original silage. This enables the use of press cake in ruminant feeding time-independently from the biorefinery process and therefore, fresh press cake seems not to be a relevant feed. Re-ensiled press cake from red clover contains more CP compared to that from grass clover mixture, what is advantageous with regard to protein supply of ruminants. However, re-ensiled press cake has lower CP and ME content compared to silage which limits its use in feeding of high-yielding ruminants with high demands on nutrients.

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CONFLICT OF INTEREST STATEMENT

There is no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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