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Effect of Harvest Stage and Ensiling on Contents of Crude Nutrients, Amino Acids, Riboflavin and Secondary Plant Metabolites of Winter Catch-Crops *Vicia sativa*, *Vicia villosa* and *Vicia pannonica*

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Correspondence: Sina Stepczynski (sina.stepczynski@thuenen.de)**Received:** 8 April 2025 | **Revised:** 25 November 2025 | **Accepted:** 5 December 2025**Keywords:** conservation | cyanoalanine toxins | organic farming | pyrimidine glycosides | silage | vetch

ABSTRACT

This study compares properties of fresh and ensiled vetch forage harvested at different growth stages: bud-, flowering-, pod formation- and pod filling stage. The research focused on three vetch species—*Vicia sativa* L. (VS), *Vicia pannonica* Crantz. (VP) and *Vicia villosa* Roth. (VV)—each represented by two varieties and cultivated in Northern Germany. The forages were ensiled with buffered formic acid and *Lactobacillus plantarum* on a laboratory scale in 1 L jars for 90 days, with four replicates per variety at each harvest stage. First, the effect of maturity stage on silage properties of whole-crop vetch was studied. The crude protein (CP), amino acid (AA), ether extract, crude ash and riboflavin contents declined during maturation ($p < 0.05$), while the unfavourable secondary metabolites γ -glutamyl- β -cyanoalanine (GCA), β -cyanoalanine (BCA) and vicine were mainly present at the pod filling stage of VS. These findings suggest that earlier growth stages are preferable as forage. Secondly, the study compared silages to the respective fresh forage to evaluate the degradation of undesired secondary plant metabolites and the conserving effect on CP, AA and riboflavin during ensiling. The ensiling process conserved CP, AA and riboflavin, while GCA, BCA and vicine were broken down ($p < 0.05$) with reduction rates of 88.2%–99.8%, 64.8%–100% and 99.9%–100%, respectively. These results indicate that ensiling is an effective conservation method for vetch forage. The occurrence of undesirable microorganisms was suppressed, as evidenced by the absence or minimal presence of butyric acid, ethanol and ammonia-N.

1 | Introduction

In organic production systems, one of the main challenges is meeting the demand for local feed resources rich in protein and vitamin B₂ (riboflavin), especially for monogastric animals (Witten and Aulrich 2019). Forage legumes, like vetch, provide protein-rich (Friman et al. 2021; Rebolé et al. 2001) and riboflavin-rich roughage (Chupakhina and Maslennikov 2004; Friman et al. 2021).

Vetch species and varieties differ in their agronomic characteristics as well as in their composition. Out of numerous species, only a few can be cultivated under temperate or cool temperate conditions in Northern Europe (Mihailović et al. 2006). Besides some varieties of *Vicia sativa* (VS), which tolerate low temperatures and frost (Firincioglu et al. 2009), vetch species that are commonly known for their adaptation to cold winter climates are *Vicia villosa* (VV) (Thurston et al. 2022) and *Vicia pannonica* (VP) (Stein et al. 2023).

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Forage derived from vetch is a nutritious feedstuff, with a crude protein (CP) content that can exceed 200 g kg⁻¹ of the dry matter (DM) (Badrzadeh et al. 2008; Georgieva et al. 2016; Rebolé et al. 2001). It can also have high proportions of available amino acids (AA) and, like grain legumes, a favourable AA profile for monogastric nutrition, with especially high proportions of leucine, isoleucine and threonine compared to organically cultivated cereals (Alzueta et al. 2001; Rebolé et al. 2001; Witten et al. 2020). Furthermore, similar to grass-red clover silage, which contains 5.4–7.6 mg riboflavin kg⁻¹ DM (Witten and Aulrich 2019), vetch silage may serve as a source of riboflavin.

The harvest stage is an important factor affecting the quality of animal feed, as it influences DM content, CP content and nutritive value (Çetin and Turk 2016; Qu et al. 2022; Rebolé et al. 2001). While DM yield, CP yield, neutral detergent fibre (NDF) content, acid detergent fibre (ADF) content and lignin content increase with maturity, CP content decreases (Çetin and Turk 2016; Rebolé et al. 2001). In general, the content of individual AA in VS is higher at early growth stages than at pod formation or full pod filling stage (Rebolé et al. 2001). In addition to these beneficial ingredients, vetch seeds, particularly those of VS, contain several unfavourable secondary plant metabolites (SPM). These include the neurotoxic compounds γ -glutamyl- β -cyano-L-alanine (GCA) and β -cyano-L-alanine (BCA) (Megias et al. 2014) as well as pyrimidine glycosides (vicine and convicine), which are characteristic of the *Vicia* genus, including *Vicia faba* (Farran et al. 2002; Tacke 2023). Enneking (1994) reviewed reports of negative effects from feeding fresh whole vetch plants rather than seeds, indicating the possible presence of these antinutritive substances and neurotoxins in vetch forages. However, their contents in vetch forages remain unknown and may depend on the variety, species, growth stage and conservation method.

Ensiling preserves several nutrients and has been shown to reduce antinutritional and neurotoxic SPM (Aulrich and Böhm 2024; Pahlow et al. 2003). This makes it a promising conservation method for whole vetch plants as feedstuff. The amount of heat-stable GCA can be reduced by mild acid hydrolysis (Aulrich and Böhm 2024; Enneking and Wink 2000). The degradation of pyrimidine glycosides can be achieved by fermentation with lactic acid bacteria or formic acid (Aulrich and Böhm 2024; Rinne et al. 2020). Additionally, the dark, compacted, acidic and oxygen-free environment of silage storage offers a high preservation potential for vitamins such as riboflavin, which is sensitive to light and high temperatures (Farrer and Macewan 1954).

In general, ensiling legumes is more challenging than ensiling grasses due to their higher buffering capacity (BC) (McDonald and Henderson 1962). Vetches, in particular, exhibit low DM contents at early vegetation stages, a high BC and low water-soluble carbohydrate contents. These contribute to a low fermentation coefficient (FC) (Schmidt et al. 1971; Weissbach 1966). Due to high CP content and high BC, legumes are susceptible to proteolysis (Martens et al. 2019; McDonald and Henderson 1962), which leads to a loss of AA (Winters et al. 2001), valuable for livestock nutrition (Partanen et al. 2013). However, proteolysis during ensiling can be inhibited by selected silage additives,

which rapidly lower the pH in wet silage (moderate target DM content of 250–350 g kg FM) and are, therefore, recommended (Turan 2020).

Despite the nutritional potential of vetch forages, it remains unclear how effectively different vetch species at different growth stages can be ensiled, aiming at a wet silage for pig feeding using a combination of formic acid and lactic acid bacteria. Furthermore, the nutritive value of the resulting wet vetch silage needs to be assessed in terms of nutritive, antinutritive and toxic compounds.

Therefore, the objectives of the present study were to evaluate:

- a. whether forages of different vetch species and varieties can be successfully ensiled using the additive combination of formic acid and *Lactobacillus plantarum*;
- b. the impact of ensiling on the contents of crude nutrients, AAs, riboflavin and selected secondary metabolites compared to the fresh forage; and
- c. the influence of vetch species and harvest stage on the overall nutrient composition of whole-plant vetch silages.

2 | Material and Methods

2.1 | Cultivation Characteristics

The field trial was carried out at the experimental station of the Thünen Institute of Organic Farming in Northern Germany (40 m above sea level [ASL], 53.76667° N, 10.51667° E), which operates in compliance with Regulation (EU) 2018/848 for organic farming (EC No 889/2008 2008; EU 2018/848 2018). This study was part of a 3-year cultivation trial. Three vetch species—hairy vetch (VV), Hungarian vetch (VP) and common vetch (VS)—were sown in pure stands at a sowing rate of 250 seeds m⁻² and a sowing depth of 3–4 cm on 11 October 2021. Each vetch species was represented by two varieties (VV: cv. Latigo and Villana; VP: cv. Beta and Detenicka; VS: cv. Carpure and Rubis) in a randomised complete block design with four replications (4 × 6 units with 25 m² plots).

The main soil type was sandy loam (18% clay, 39% silt, 43% sand) with a pH of 6.3 and a N_{\min} content of 28.3 kg ha⁻¹ (measured at the time of sowing, depth 0–90 cm). The average temperature from January 2021 to December 2022 was 9.8 °C, with a cumulative precipitation of 595 mm (DWD (Deutscher Wetterdienst) 2024) (Figure 1). Between 1986 and 2022, the location exhibited a mean temperature of 9.2 °C and 686 mm of cumulative precipitation.

2.2 | Harvest

Phenological development of the forages was continuously monitored using the BBCH scale (Federal Plant Variety Office 2000). Forage was harvested at four harvest stages:

- Bud stage (BBCH: 51–59)
- Flowering stage (BBCH: 65)

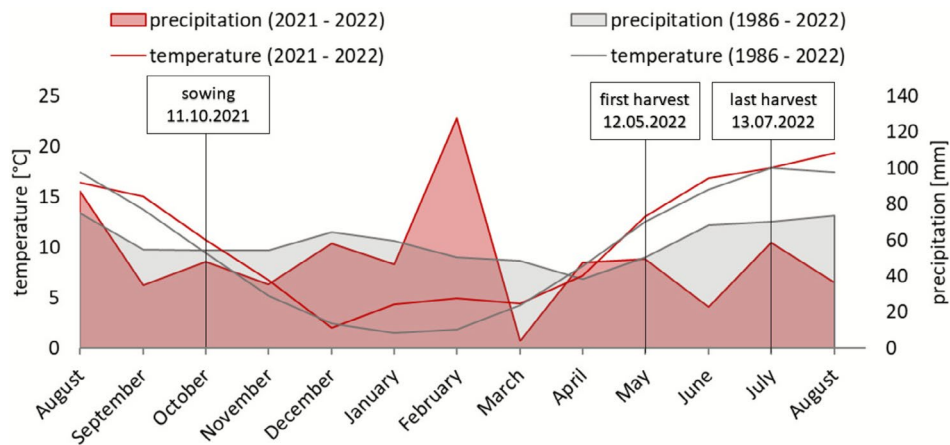


FIGURE 1 | Monthly average temperature and accumulated precipitation during the forage growing period.

TABLE 1 | Sowing dates (S), harvest dates (H) and growing days after sowing (GDAS) of the vetch forages at four harvest stages.

S	Spe-cies	Variety	Bud stage		Flowering stage		Pod formation stage		Pod filling stage	
			H	GDAS	H	GDAS	H	GDAS	H	GDAS
11.10.21	VP	Beta	16.05.22	217	24.05.22	225	14.06.22	246	06.07.22	268
11.10.21	VP	Detenicka	18.05.22	219	24.05.22	225	14.06.22	246	06.07.22	268
11.10.21	VS	Carbure	12.05.22	213	18.05.22	219	31.05.22	232	13.06.22	245
11.10.21	VS	Rubis	16.05.22	217	20.05.22	221	31.05.22	232	13.06.22	245
11.10.21	VV	Latigo	23.05.22	224	07.06.22	239	23.06.22	255	13.07.22	275
11.10.21	VV	Villana	23.05.22	224	07.06.22	239	23.06.22	255	13.07.22	275

- Pod formation stage (BBCH: 69–71)
- Pod filling stage (BBCH: 71–79).

The harvest by maturity stage resulted in different harvest dates for each variety (Table 1).

At each stage, approximately 6 kg of fresh matter (FM) was harvested per plot at a cutting height of 6–8 cm above ground using a cutter bar mower (Agria 5400, Agria-Werke GmbH, Möckmühl, Germany). Vetch biomass from the first cut was separated from weeds, whereas subsequent cuts were analysed without separation due to low weed pressure. Fresh vetch forage samples were collected immediately for DM content determination.

2.3 | Ensiling

The BC was determined in advance, according to Weißbach (1992), at the Julius Kühn Institute (Institute for Crop and Soil Science, Braunschweig, Germany) using plant samples from the previous year (2021) to calculate the amount of formic acid required for ensiling. Due to time constraints and in order to determine an equivalent formic acid dosage for all samples, material of one variety per species (VP: Beta, VS: Rubis, VV: Latigo) at two harvesting stages (flowering and pod formation stage) was selected for BC calculation.

Before ensiling, harvested material (6 varieties × 4 repeated blocks × 4 cuts = 96 samples) was placed on a surface dryer with a drying time between 0.0 and 5.7 h to achieve a DM content of about 25%. A microwave method was used to determine the DM content immediately (Losand and Waldmann 2003). Wilted forage material was processed with a cutter (SM 65 STL, K + G Wetter GmbH, Biedenkopf, Germany) at 3000 rpm for two rotations of the cutter bowl, then homogenised and mixed again at 2500 rpm for two rotations of the cutter bowl. To analyse and determine the DM content, wilted vetch samples were collected to assess the impact of subsequent ensiling.

Subsequently, 10 mL of buffered formic acid (Amasil NA, BASF, Ludwigshafen am Rhein, Germany) per kg vetch biomass was added to each sample and mixed to lower the pH to 4.0. The formic acid dosage was chosen based on BC, low target DM and low sugar content (Pieper et al. 2007), to produce stable silage, with the aim of conserving valuable nutrients and decreasing unfavourable or toxic plant metabolites. Each sample of the acidified silage was inoculated with 1.2 mg of facultative heterofermentative lactic acid bacteria (BIO-SIL, Dr. Pieper Technologie- und Produktentwicklung GmbH, Neuruppin, Germany) per kg of vetch biomass (3.6×10^8 CFU g^{-1} FM as recommended on the product description sheet) and compressed (136.1–246.7 kg DM m^{-3}) in two 1 L jars (J. WECK GmbH & Co. KG, Wher-Öflingen, Germany). Amasil NA and

BIO-SIL are approved for organic farming under Regulation (EU) 2018/848 and Regulation (EU) 2021/1165.

The pH was measured using a pH meter (PH 539 brand WTW; Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim, Germany) at three time points: on Day 0 after inoculation, on Day 3 (small 50g vacuum pack silos) and on Day 90, when the jars were opened. After 90 days of storage at a temperature of 21°C–24°C, silage samples were extracted and material from the two jars containing the same sample was homogenised.

2.4 | Sample Preparation

To determine the organic acid profile and alcohol contents of the silage samples, fresh silage material was taken during jar opening (Day 90), immediately frozen and stored at –21°C for later analysis. The DM content of both fresh forages and silages was determined using an industrial oven (type CD, Caldatrac Industrieofenbau GmbH & Co. KG, Höchheim, Germany), where biomass was dried to a constant weight (24 h, 105°C) (Federal Plant Variety Office 2000). For crude nutrient analysis, samples were dried at 40°C and ground to pass through a 1 mm sieve (Foss, CT 293 Cyclotec, Foss GmbH, Hamburg, Germany). Another portion of the samples was freeze-dried (Alpha 1-4 LSCplus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and ground through a 0.5 mm sieve (Foss, CT 293 Cyclotec, Foss GmbH, Hamburg, Germany) for the determination of AA, riboflavin and SPM content.

2.5 | Analytical Procedure

The organic acid profile and alcohol content of the silage samples were determined using high-performance liquid chromatography (HPLC) according to Siegfried et al. (1984), performed at the Julius Kühn Institute (Institute for Crop and Soil Science, Braunschweig, Germany). During the drying process of the silage, volatile organic components, including formic acid, evaporated. To account for these losses, the uncorrected DM content DM_u of the ensiled material was corrected. An equation according to Weißbach and Strubelt (2008) was used and adapted by incorporating formic acid, assuming a mean volatility of 92.04% across all pH classes based on Huida et al. (1986) (Stepczynski et al. 2025).

$$DM_c = DM_u + (1.05 - 0.059 \text{ pH})LFA + 0.9204 FA^* + 0.08 LA + 0.77PD + 0.87 BD + 1.00 OA \left[\frac{\text{g}}{\text{kg FM}} \right]$$

where *LFA* denotes total low fatty acids (C_2 – C_6), *FA** formic acid, *LA* lactic acid, *PD* 1,2-propanediol, *BD* 2,3-butanediol, and *OA* total other alcohols (C_2 – C_4).

Crude nutrient composition was analysed following Regulation EC No 152/2009 (EC No 152/2009 2009) and VDLUFA (2012). CP content was calculated from nitrogen analysis using a conversion factor of 6.25. Crude fibre (CF) content was determined based on the method of Van Soest et al. (1991).

AA content analysis followed Regulation (EC) No 152/2009, adjusted with a pre-column derivatisation with 6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (Cohen and Michaud 1993; EC No 152/2009 2009). Separation was performed using HPLC (Infinity 1260, Agilent Technologies Deutschland GmbH, Waldbronn, Germany) with a C18 column (3 µm Gemini NX C18 150×4.6, Phenomenex Ltd., Aschaffenburg, Germany) in combination with a fluorescence detector (FLD) (Infinity 1260, Agilent Technologies Deutschland GmbH, Waldbronn, Germany; Witten et al. 2020). Methionine was measured as methionine sulfone and cysteine as cysteine acid. Riboflavin content was analysed using HPLC-FLD (Agilent Technologies Deutschland GmbH, Waldbronn, Germany) with separation on a C18 column (Kinetex 5 µm XB-C18 150×4.6 mm, Phenomenex Ltd., Aschaffenburg, Germany) (DIN EN 14152:2014-08, 2014; Witten and Aulrich 2018). The pyrimidine glycoside vicine was analysed according to Pulkkinen et al. (2015) using HPLC attached to a diode array detector (wavelength: 273 nm). The separation was carried out on a C18-column (Kinetex 5 µm XB-C18 150×4.6 mm, Phenomenex Ltd., Aschaffenburg, Germany) (Pulkkinen et al. 2015). A mass selective detector was used to confirm the vicine peak. The determination of BCA and GCA was conducted according to Thavarajah et al. (2012) using HPLC and mass spectrometric detection (Agilent Technologies Deutschland GmbH, Waldbronn, Germany). The separation was performed on a C18 column (Kinetex 5 µm XB-C18 150×4.6 mm, Phenomenex Ltd., Aschaffenburg, Germany), followed by detection of the specific masses using a mass spectrometer, $[M + H]^+$ m/z 115.1 for BCA and $[M + H]^+$ m/z 244.22 for GCA.

2.6 | Calculations to Assess Ensilability and Ensiling Success

FC, as described by Schmidt et al. (1971), was used to assess the fermentability of different vetch species:

$$FC = DM (\%) + \left(8 \cdot \frac{\text{sugar}}{BC} \right)$$

Fermentation loss (FL) was determined based on the mass difference between the start and end of fermentation (ΔM), relative to the initial DM weight (weigh-in DM) and corrected for dissolved CO_2 according to Kaiser and Weißbach (1989).

$$FL [\%] = 100 \times \left(\frac{\Delta M (\text{g})}{\text{weigh-in DM (g)}} \right) + 2.5$$

2.7 | Statistical Analysis

All statistical analyses were performed using R in combination with R Studio (v4.4.0; Posit team 2024; R Core Team 2024). Analysis of the target variables in the ensiled material was carried out using a model considering repeated measurements. Since samples were taken on the same plots over time, the analytical results were likely to be serially correlated. The nlme package was used to analyse the repeated measurements (Pinheiro et al. 2023; Pinheiro and Bates 2000). The corExp() correlation structure was selected because the intervals between harvest stages were not necessarily equal (Piepho and Edmondson 2018). Normal distribution of the residuals and the

TABLE 2 | Composition of fresh vetch forage from three species of *Vicia* depending on harvest stage.

	Unit	Harvest stage	<i>V. pannonica</i> (n = 8)		<i>V. sativa</i> (n = 8)		<i>V. villosa</i> (n = 8)	
			Mean	SD	Mean	SD	Mean	SD
Dry matter (DM)	Fresh matter (FM), g kg ⁻¹	Bud	137	12	136	12	126	10
		Flowering	147	18	129	14	137	8
		Pod formation	199	18	158	25	201	18
		Pod filling	262	14	257	11	310	23
DM after wilting	FM, g kg ⁻¹	Bud	176	6	199	14	212	11
		Flowering	185	6	191	13	199	20
		Pod formation	195	14	237	23	202	19
		Pod filling	264	11	280	11	323	11
Crude protein	DM, g kg ⁻¹	Bud	210 ^{Ba}	16	279 ^{Cc}	13	253 ^{Db}	14
		Flowering	194 ^{Ba}	21	271 ^{Cc}	12	226 ^{Cb}	10
		Pod formation	161 ^{Aa}	11	227 ^{Bc}	11	191 ^{Bb}	14
		Pod filling	150 ^{Aa}	10	179 ^{Ab}	18	161 ^{Aa}	8
Sugar	DM, g kg ⁻¹	Bud	46 ^{Bb}	9	34 ^{Aa}	14	29 ^{ABa}	7
		Flowering	26 ^{Aa}	6	28 ^{Aa}	5	31 ^{Ba}	4
		Pod formation	35 ^{Aa}	6	50 ^{Bb}	6	31 ^{Ba}	4
		Pod filling	28 ^{Aa}	6	86 ^{Cb}	8	21 ^{Aa}	5
Starch	DM, g kg ⁻¹	Bud	29 ^{Aa}	7	15 ^{Aa}	5	18 ^{Aa}	3
		Flowering	23 ^{Aa}	5	8 ^{Aa}	9	21 ^{Aa}	4
		Pod formation	56 ^{Ba}	10	107 ^{Bb}	27	69 ^{Ba}	16
		Pod filling	131 ^{Ca}	15	169 ^{Cb}	19	145 ^{Ca}	21
Fermentation coefficient ^a		Bud	222		239		234	
		Flowering	210		223		223	
		Pod formation	229		296		226	
		Pod filling	292		382		339	

Note: Means followed by a different capital letter show significant differences between harvest stages within a species according to the Tukey HSD post hoc test. Means marked with a different lowercase letter show significant differences between species within a given harvest stage; $p < 0.05$.

^aMean values for buffering capacity were 8.1, 6.8 and 10.2 g lactic acid (LA) 100 g⁻¹ DM for *Vicia pannonica*, *Vicia sativa* and *Vicia villosa*, respectively and were used to estimate the fermentation coefficient.

homogeneity of variance were assessed (Hebbali 2020; Lüdecke et al. 2021). The crude nutrient composition, as well as the contents of AA, riboflavin and secondary metabolites in silages, were evaluated by ANOVA. The model included harvest stage, variety and their interaction (harvest stage × variety) as the main effects. The model-based mean estimates (adjusted least-square means), along with their appropriate standard errors and confidence limits, were calculated using the emmeans package (Lenth 2024). Multiple comparisons of means were performed with a Tukey HSD-test at a significance level of 0.05, with a compact letter display.

To determine the effect of ensiling, the model was expanded to include the fixed factor treatment (fresh vs. ensiled). If the null hypothesis (H_0 : no differences between ensiled and fresh forage) was rejected, a more detailed analysis was conducted using the

adjusted repeated measurement model and multiple comparisons. Multiple comparisons of means were performed at a significance level of 0.05, applying a Bonferroni cross-adjustment for various sets of comparisons.

3 | Results

3.1 | Characteristics of Fresh Vetch Forage

The DM content of the fresh material of the three vetch species increased over the cultivation period and exceeded 200 g kg⁻¹ FM at the final harvest stage (Table 2). Although wilting increased the DM content at the bud and flowering stages, it did not lead to contents above 250 g kg⁻¹ FM in any species.

The CP content of the vetches decreased by an average of 54 g CP kg⁻¹ DM from the bud to pod formation stage ($p < 0.05$). VS and VV consistently showed higher CP contents than VP ($p < 0.05$), except at the pod formation stage. VS had the highest mean CP content at the bud and flowering stages ($p < 0.05$). While VS had the highest CP content, the BC was lower than that of VP and VV. Despite wilting, the FC was low. The sugar content of VS increased significantly at the pod filling stage ($p < 0.05$). The highest available sugar contents were observed at the pod filling stage in VS, with mean values of 81 ± 1 g kg⁻¹ DM for Carbure and 91 ± 9 g kg⁻¹ DM for Rubis ($n = 4$) (Table S1). The description ($n = 4$) could be added either after the average values or after the variety (uniformly).

3.2 | Characteristics of Ensiling

After the application of formic acid (ensiling duration of 0 days), the forage immediately reached a pH ranging from 3.8–4.9 on Day 0 of ensiling (Figure S1). After 90 days of storage, the pH was below 4.5 in all varieties (Table 3). While the harvest stage affected the pH value after 90 days, the variety of vetch had no significant influence.

A significant effect of the interaction between harvest stage and variety was observed for all analysed fermentation products, with the exception of the pH value after 90 days of ensiling (Table 3) and the butyric acid content (Table S2).

The content of potentially volatile fermentation products, except for formic acid, was low across all ensiled samples. Formic acid accounted for an average of $84.9\% \pm 5.5\%$ of the total organic acids and alcohols. The majority of the short-chain fatty acids, which are known to be volatile, consisted of acetic acid. Propionic acid was not detected in ensiled VS and was only found in one harvest of VV. The ensiled varieties exhibited none to low propionic acid and butyric acid contents. The alcohols in the ensiled vetch from VS and VP predominantly consisted of ethanol. The ensiled VS varieties had the highest ethanol contents from the bud to flowering stage (7.3 ± 3.0 – 7.6 ± 1.2 g kg⁻¹ DM). The ensiled Villana (VV) samples from the first harvest exhibited significantly higher FLs compared to all other samples ($p < 0.05$).

3.3 | Effect of Ensiling and Harvest Stage on the Chemical Composition of Vetch Species

3.3.1 | Crude Nutrients

The CP content was influenced by the harvest stage (Figure 2). An early harvest stage resulted in protein-rich silages. Carbure (VS) achieved the highest CP content across all harvest stages, both in the fresh and the ensiled vetch. The CP content of VP, VS and VV decreased from bud to pod formation stage by 38.7 ± 10.5 , 45.6 ± 10.3 and 65.3 ± 12.3 g CP kg⁻¹ DM, respectively. Ensiling decreased the mean CP content by 7.3% ($p = 0.004$). However, significant differences between the CP content of fresh and ensiled material were observed only twice in the bud stage and once each in the flowering and pod formation stages (marked with asterisks in Figure 2).

The harvest stage and variety also influenced the other crude nutrients (Table S3). The ether extract (EE) content decreased with increasing maturity. The interaction between treatment, harvest stage and variety affected the EE content of the ensiled vetch ($p < 0.05$). Both ensiled VS varieties, in particular, showed significantly higher EE contents at the bud stage than in fresh forage.

Across all harvest stages, VS had the lowest CF content, which remained below 250 g kg⁻¹ DM in both fresh and ensiled material. The CF content of ensiled VS Carbure did not increase significantly from bud to pod filling stage. In ensiled VP and VV, the CF increased significantly ($p < 0.05$) by 36.5 g kg⁻¹ DM and 56.2 g kg⁻¹ DM, respectively.

The crude ash (CA) content of the ensiled vetch decreased over maturation and was significantly lower at the pod filling stage than at the other harvest stages in all species.

3.3.2 | AA Pattern

As the vetch plants matured, the AA contents of the ensiled vetch decreased ($p < 0.05$) (Figure S3). The ensiled Carbure (VS) consistently had the highest content of total AA across all harvest stages (212.9 ± 3.2 g kg⁻¹ DM, 204.4 ± 8.6 g kg⁻¹ DM, 172.2 ± 6.1 g kg⁻¹ DM, 147.9 ± 5.0 g kg⁻¹ DM). Ensiling reduced the mean total AA content of fresh vetch by 10.6 g kg⁻¹ DM or 6.5% ($p = 0.047$). In both fresh and ensiled vetch forage of VV, VS and VP, aspartic acid (25.5 ± 5.8 g kg⁻¹ DM, $n = 96$; 24.1 ± 5.4 g kg⁻¹ DM, $n = 96$) and glutamic acid (19.1 ± 3.8 g kg⁻¹ DM, $n = 96$; 17.1 ± 3.5 g kg⁻¹ DM, $n = 96$) were the most abundant AA, with aspartic acid consistently having the highest content. Cysteine, methionine and histidine were least abundant in all vetch forages, with cysteine consistently having the lowest content (1.9 ± 0.3 g kg⁻¹ DM in fresh vetch; 1.3 ± 0.2 g kg⁻¹ DM in ensiled vetch).

The contents of AA were not always affected by ensiling. Among the essential and semi-essential AA, cysteine was reduced across all varieties and harvest stages during ensiling (variety \times treatment $p = 0.008$). Ensiling had no effect on the content of valine, isoleucine, leucine, phenylalanine or tyrosine (Figure 3a).

The proportion of total AA in CP decreased only slightly in VS with increasing maturity (Figure S2). The proportion of cysteine in the total AA content of the vetches showed the greatest negative deviation from fresh to ensiled forage, with a reduction of 24.9%. The proportion of valine and tyrosine in the total AA content increased in ensiled vetch. At later harvest stages, the proportion of cysteine in the total AA increased in the silage samples, while the proportion of leucine, methionine, tyrosine and phenylalanine in the total AA decreased (Figure 3b).

3.3.3 | Riboflavin Content

The harvest stage and variety influenced the riboflavin content in the ensiled vetch (variety \times harvest stage $p < 0.001$) (Figure 4; Table S3). In ensiled vetch, riboflavin content ranged from 13.2 ± 1.7 to 12.1 ± 1.7 mg kg⁻¹ DM from the bud to flowering stage. VS had the highest riboflavin content, which was

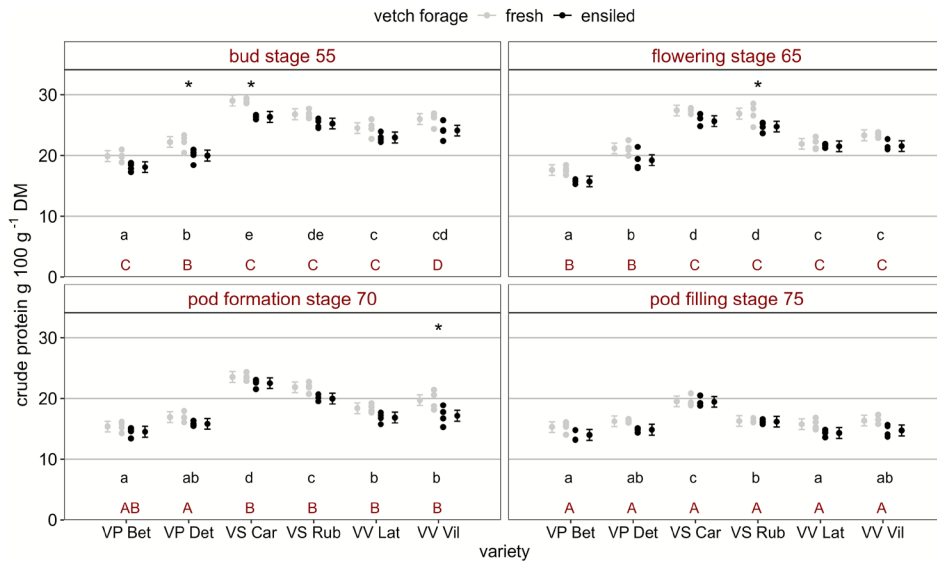


FIGURE 2 | Crude protein content of fresh and ensiled forage of the different vetch species *Vicia pannonica* (VP), *Vicia sativa* (VS) and *Vicia villosa* (VV) of the varieties Beta (Bet), Detenicka (Det), Carburne (Car), Rubis (Rub), Latigo (Lat) and Villana (Vil). Error bars with dots represent adjusted estimated marginal means with 95% confidence limits for each variety at each harvest stage. Within each harvest stage, the different black lowercase letters represent significantly different mean values of ensiled vetch between varieties according to the Tukey HSD post hoc test. For each variety, the different red capital letters indicate significantly different mean values of ensiled vetch between harvest stages. *Statistically significant differences between mean values of fresh and ensiled material at each harvest stage and for each variety.

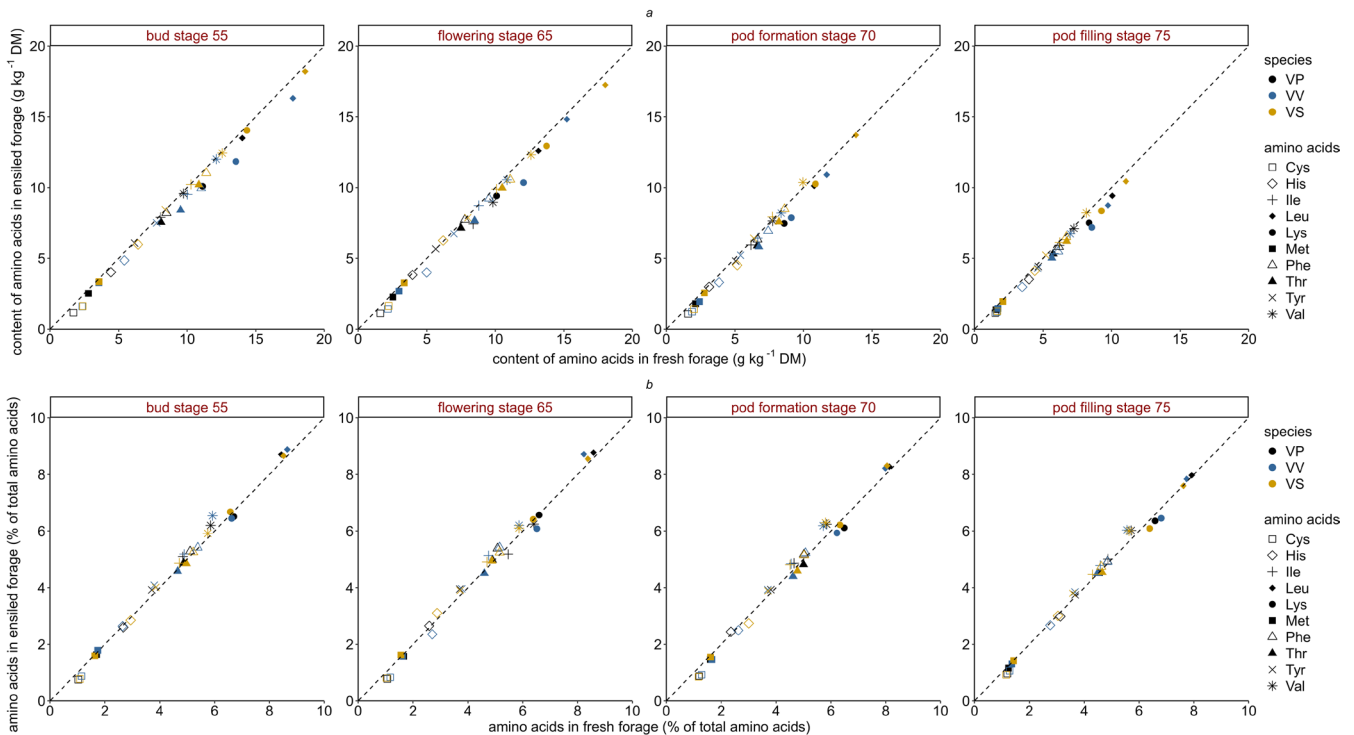


FIGURE 3 | Mean content of essential and semi-essential amino acids (a) in g kg^{-1} DM and (b) in % of total amino acids in ensiled forage of the vetch species *Vicia pannonica* (VP), *Vicia sativa* (VS) and *Vicia villosa* (VV), plotted against the corresponding values of fresh vetch forage.

significantly higher than that of VP during the first three harvest stages ($p < 0.05$). The riboflavin content of VV decreased after the bud stage, while in VS and VP, it decreased after the flowering stage ($p < 0.05$). The greatest reduction in riboflavin content was observed in VV from the bud to pod filling stage (by 10.2 mg kg^{-1} DM; decrease rate = 72.9%), followed by VP (by

5.4 mg kg^{-1} DM; decrease rate = 48.9%) and VS (by 6.5 mg kg^{-1} DM; decrease rate = 44.6%). Among the varieties, Carburne exhibited a lower decrease (39.3%) than Rubis (50.2%) over the vegetation period. Significant differences between fresh and ensiled forage material were observed during the pod formation and pod filling stages ($p < 0.05$). While the average degradation

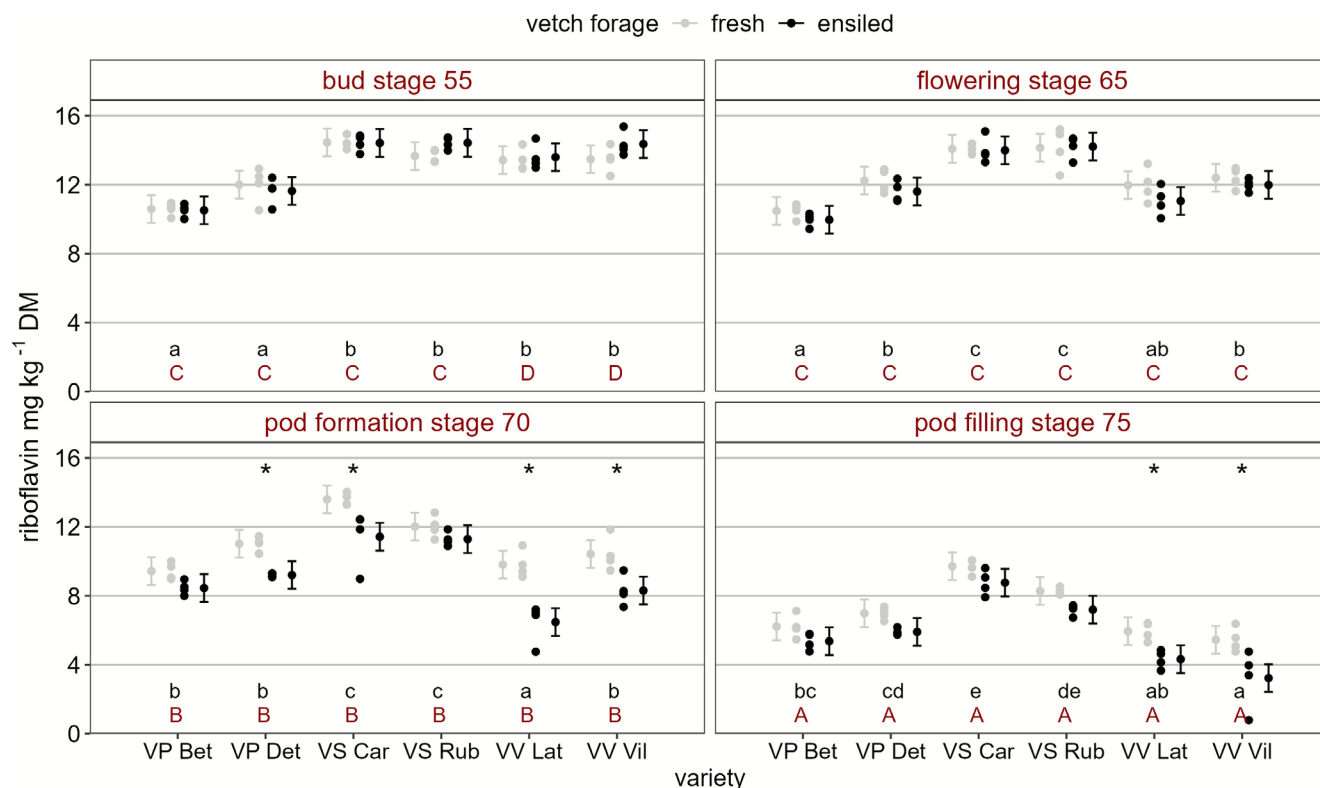


FIGURE 4 | Riboflavin content of fresh and ensiled forage from the different vetch species *Vicia pannonica* (VP), *Vicia sativa* (VS) and *Vicia villosa* (VV) of the varieties Beta (Bet), Detenicka (Det), Carure (Car), Rubis (Rub), Latigo (Lat) and Villana (Vil). Error bars with dots represent adjusted estimated marginal means with 95% confidence limits per variety for each harvest stage. For each harvest stage separately, different black lowercase letters represent significantly different mean values between varieties according to the Tukey HSD post hoc test. For each variety, different red capital letters represent significantly different mean values between the harvest stages. *Statistically significant differences between the mean values of fresh and ensiled material at each harvest stage and for each variety.

rate between fresh and ensiled forage remained below 5% at the bud and flowering stages ($-1.6\% \pm 4.1\%$, $n=24$; $3.4\% \pm 4.9\%$, $n=24$), it increased to an average of $17.1\% \pm 11.0\%$ ($n=24$) and $20.3\% \pm 15.9\%$ ($n=24$) at the pod formation and pod filling stages, respectively.

3.3.4 | Content of Secondary Plant Metabolites

The content of GCA, BCA and vicine varied with variety and harvest stage (Table 4). None of the VV varieties contained any of the analysed toxic secondary metabolites. Neither VV nor VP contained GCA; however, GCA was found in fresh VS forage at all harvest stages, with contents increasing continuously from the bud stage to the pod-filling stage.

BCA was undetectable in VV and VP but was found in fresh VS forage at the pod-filling stage. In ensiled VS forage, lower contents were observed.

The pyrimidine glycoside vicine was detected in VS at the stages of pod formation and pod filling. Fresh VS forage contained vicine, with Rubis (VS) having significantly lower vicine contents than Carure (VS). In VP, vicine was present during the pod filling stage. However, vicine was degraded during ensiling

and was only detected in trace amounts in the ensiled vetch ($0.0-0.1 \text{ mg kg}^{-1} \text{ DM}$).

4 | Discussion

4.1 | Characteristics of Fresh Vetch Forage

Due to time constraints and technical limitations, the drying process was less efficient than expected, resulting in more challenging ensiling conditions during the first three harvest stages. However, since excessive drying and long drying phases can lead to crumbling losses of leaves (Hettasch 1995), which contain higher amounts of CP and less CF than the stems (Huang et al. 2019), gentle drying is considered beneficial.

Badrzadeh et al. (2008) reported a higher CP content of $226 \text{ g kg}^{-1} \text{ DM}$ for VP at the flowering stage. The CP content of VV and VS in the present study exceeded the content reported by Georgieva et al. (2016) and Rebolé et al. (2001), respectively. These deviations may be due to different growing and harvesting conditions or to differences between varieties. Compared to other leguminous roughages, vetch achieves CP contents similar to those of alfalfa ($221 \text{ g kg}^{-1} \text{ DM}$) (Lin et al. 2023) and, depending on the species, may even exceed them. This makes vetch an appealing feedstuff.

TABLE 4 | Estimated marginal means of γ -glutamyl- β -cyano-L-alanine (GCA), β -cyano-L-alanine (BCA) and vicine content in fresh and ensiled forage from the vetch species *Vicia sativa* (VS) and *V. pannonica* (VP), depending on the harvest stage.

		Harvest stage	Fresh				Ensiled			
			VP		VS		VP		VS	
			Beta	Detenicka	Carbure	Rubis	Beta	Detenicka	Carbure	Rubis
GCA	Dry matter (DM), mg kg ⁻¹	Bud	0.0 ^{Aa}	0.0 ^{Aa}	94.2 ^{Ab}	115.3 ^{Ac}	0.0 ^{Aa}	0.0 ^{Aa}	9.6 ^{Bb*}	9.2 ^{Cb*}
		Flowering	0.0 ^{Aa}	0.0 ^{Aa}	84.2 ^{Ab}	108.4 ^{Ac}	0.0 ^{Aa}	0.0 ^{Aa}	7.6 ^{Bb*}	7.4 ^{Cb*}
		Pod formation	0.0 ^{Aa}	0.0 ^{Aa}	400.2 ^{Bc}	152.1 ^{Bb}	0.0 ^{Aa}	0.0 ^{Aa}	5.4 ^{Ab*}	5.3 ^{Bb*}
BCA	DM, mg kg ⁻¹	Bud	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}
		Flowering	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}
		Pod formation	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}
Vicine	DM, mg kg ⁻¹	Bud	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}
		Flowering	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}
		Pod formation	0.0 ^{Aa}	0.0 ^{Aa}	259.1 ^{Bc}	45.1 ^{Bb}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Aa*}
		Pod filling	59.0 ^{Bc}	37.9 ^{Bb}	1657.4 ^{Cd}	1344.5 ^{Cd}	0.0 ^{Aa}	0.0 ^{Aa}	0.0 ^{Bb*}	0.1 ^{Bc*}

Note: Different capital letters represent significantly different mean values between harvest stages within a variety of fresh or ensiled vetch. Different lowercase letters represent significantly different mean values between the varieties within a harvest stage of fresh or ensiled vetch, according to the Tukey HSD post hoc test with a significance level of 0.05.

*Behind ensiled mean values represent statistically significant differences between the mean values of fresh and ensiled material at each harvest stage and for each variety.

However, due to the high buffering capacities of 68–102 g LA kg⁻¹ DM, the vetch species are difficult to conserve, even compared to alfalfa, which has a BC of 65 g LA kg⁻¹ DM (Knezevic et al. 2011). Furthermore, ensiling was difficult due to low sugar and DM contents, which led to a low FC of < 35 in all vetch species at every harvest stage except for VS at the pod formation stage. The low FC of 25 for VS was also described by Pursiainen and Tuori (2008). The ensiling ability of the tested vetch plants was thus rather poor and the use of silage additives is advisable.

4.2 | Characteristics of Ensiling

Using formic acid and lactic acid bacteria, stable, high-quality vetch silage was produced despite the low sugar content, high BC and low DM content.

Nevertheless, due to a higher content of available sugars and presumably the activity of yeasts (Pursiainen and Tuori 2008), VS silage had a higher ethanol content compared to VV and VP. But since the content of the vetch silage in the trial did not exceed 10 g kg⁻¹ DM, ethanol production of ethyl

acetate and ethyl lactate was limited according to Weiß and Auerbach (2013). Weiß and Kalzendorf (2017) ensiled red clover and alfalfa with different additives. They reported ethanol contents comparable to those of VS silages in red clover and alfalfa silages treated with lactic acid bacteria additives. Furthermore, they observed ethanol contents comparable to those of VP and VV silages in silages produced with salt additives. One reason could be the lower sugar content and thus lower precursors for ethanol production in VV and VP. The FLs were lower than those reported by Pursiainen and Tuori (2008), which could indicate a greater reduction in FL due to increased formic acid use.

The present study confirmed that even with a low FC of < 35, the addition of 10 L t⁻¹ of formic acid, which inhibited the formation of fermentation products like lactic acid and acetic acid, also sufficiently inhibited the undesirable butyric acid production. Contrary to the findings of Pahlow et al. (2003), this trial did not confirm an increased probability of clostridial fermentation during ensiling, even with FC values below 45.

The application rate of 10 L t⁻¹ of formic acid prompted a rapid decline in pH in all samples. The lactic acid bacteria were added

after homogenising the wilted vetch biomass with the buffered formic acid to avoid pH values <4.0, which can be detrimental to the bacteria (Popova-Krumova et al. 2024). However, the lactic acid content in all silage samples was less than 4 g kg⁻¹ DM, which is low compared to the results of Pursiainen and Tuori (2008), who observed a content of 28 and 71 g kg⁻¹ DM in VS ensiled with and without 4 mL formic acid kg⁻¹ FM, respectively. These results indicate that the addition of buffered formic acid inhibited the lactic acid bacteria. Also, buffering Na⁺ can lead to salt stress and a reduction in growth rates of *Lactobacillus plantarum* (Wang et al. 2016). The addition of 10 mL buffered formic acid kg⁻¹ FM probably inhibited the development of both the desired fermentation products and the undesired metabolic products. Although the formic acid dose is quite high compared to that used by Pursiainen and Tuori (2008), it may be appropriate for larger-scale on-farm use. The bale silage (produced with the same dosage of the same additives in the same addition order) of an on-farm feed trial for pigs reached levels of 9.3 to 32.0 g lactic acid kg⁻¹ DM (Wiskandt et al. 2025). This leads to the assumption that the combination of lactic acid bacteria and formic acid worked more appropriately in larger-scale on-farm use despite the addition of high doses of formic acid. Nevertheless, a reduction and/or a single use of formic acid can achieve stable silage (Lei et al. 2024; Yan et al. 2025) and can be beneficial for economic efficiency. However, in this study and the upscale on-farm use study, the ensiling additives were primarily added to conserve valuable nutrients and effectively degrade value-reducing SPM, as well as reach a high stability of silage for use in pig feeding. Furthermore, the addition of acid can positively influence the feed intake of pigs (Hellekant and Danilova 1999; Roura et al. 2011; Roura and Tedó 2025). Future research on ensiling vetch plants should be conducted with reduced amounts of formic acid. This applies particularly from the flowering stage onwards, as the CP content and CA content decrease (Stepanova and Volovik 2021) while DM contents increase. Reducing acid additive use is also desirable due to the handling challenges farmers face.

4.3 | Effect of Ensiling on the Chemical Composition of Vetch Species

4.3.1 | Crude Nutrients

Wilting duration was determined by measuring the DM content of fresh vetch forage at each harvest stage and adjusting surface drying to produce wet silage that serves as an attractive roughage for pigs (Jeroch et al. 1991; Olsen et al. 2000). However, the target DM could not be achieved for all forage samples due to technical difficulties in surface drying and heterogeneous material.

Due to significantly decreasing CP, EE and CA contents as well as significantly increasing CF contents, the feed value of vetch biomass generally decreased with the advanced growth of vetch plants. However, VS and especially VS Carbure had low CF contents throughout maturation, indicating feeding advantages, as also indicated by a high total tract digestibility of CP of 55%–70% in fattening pigs (Wiskandt et al. 2025). Due to the low sugar and DM contents and the high BC of vetches, there is a higher risk of proteolysis during the ensiling and storage process (McDonald

and Henderson 1962). This can lead to a significantly decreased CP content in legume silage (Kara and Sürmen 2023). However, in the present study, the addition of formic acid rapidly lowered the pH and effectively inhibited the proteolysis in all vetch species, consistent with the findings of McKersie (1985).

At the bud and flowering stages, ensiled VP had a CP content comparable to that of red clover and alfalfa silage (Kornfelt et al. 2013; Lin et al. 2023). Ensiled VS and VV contained CP content similar to white clover silage (Kornfelt et al. 2013). The decreasing CA content could be related to a decline in or dilution, according to DM accumulation, of minerals and trace element levels with advancing maturity (Tan et al. 2003; Türk et al. 2010). The observed decrease in CA content may also indicate a low contamination rate during growth, despite the increased risk of lodging with maturity and greater plant height (Uzun et al. 2005).

4.3.2 | AA Profile

Early harvest stages led to significantly higher AA contents in the silage samples, which aligns with findings observed by Rebolé et al. (2001). According to Pursiainen and Tuori (2008), limited protein degradation and reduced ammonia-N content indicate an effective ensiling. Winters et al. (2001) stated that legume ensiling can lead to a reduction in the content of individual AA, including cysteine, methionine, aspartic acid, glutamic acid, phenylalanine, lysine and arginine. The general conservation of AA observed in this study suggests that the ensiling process, under the applied conditions, did not result in substantial degradation of most AA. The AA profile of the vetches was similar to that found by Rebolé et al. (2001). Compared to organically cultivated cereals, arginine and histidine contents were similar, while the contents of lysine, threonine, tyrosine and valine were higher (Witten et al. 2020). Contents of threonine and valine in vetch silages of all growth stages and methionine at bud and flowering stages were even comparable to those of grain legumes (Witten et al. 2020). Although the observed range was wide and dependent on species and harvest stage, the levels of lysine and methionine were similar to those of previous studies with vetches (Rebolé et al. 2001). Blume et al. (2021) reported lysine and methionine contents in fresh alfalfa across different harvest stages of 7.5 ± 1.0–8.4 ± 1.3 g kg⁻¹ DM and 2.0 ± 0.4–2.5 ± 0.7 g kg⁻¹ DM, respectively. These contents are comparable to vetch silage from later harvest stages, while vetch silage from bud and flowering stages can serve as a valuable source of lysine and methionine in animal feed, although cysteine contents were reduced during ensiling.

Regarding high AA contents, the VS varieties are more favourable to the VP and VV varieties. Early harvest stages, like the bud and flowering stages, are favourable to later harvest stages.

4.3.3 | Riboflavin Content

At the bud stage, the riboflavin contents even exceeded those reported for regionally cultivated grass-red clover silage (Witten and Aulrich 2019). In 75% of the laboratory silages, riboflavin was successfully preserved. This may be attributed to the dark,

compacted, acidic and oxygen-free storage conditions (Ahmad et al. 2004; Farrer and Macewan 1954). The instability of riboflavin is low at a pH of 4–6, aligning with the silage pH values in the trial (Ahmad et al. 2004). In addition, the photostability of riboflavin in an acidic medium is twice as high as in a neutral medium (Astanov et al. 2016). Since riboflavin is sensitive to light exposure (Farrer and Macewan 1954), surface drying the harvested material indoors (protected from light) rather than in the field may have helped to reduce losses. A lower target DM can be a critical factor in maintaining high riboflavin levels, as it requires a shorter drying time.

4.3.4 | Content of Secondary Plant Metabolites

None of the VV varieties contained any of the analysed SPM. However, the toxicity of VV has been attributed to L-canavanine by Enneking (1994) and Enneking and Wink (2000) and to cyanamide by Kamo et al. (2015), which we did not analyse in this study. Thus, the absence of the analysed SPM does not confirm the absence of toxicity.

Among the vetch species, VS forage had the highest levels of the analysed SPM. As traces of GCA were detected in phenological stages without pods or seeds, its presence in other parts of the plant (leaves or stems) is likely, as traces were also found in phenological stages without pods or seeds. However, an increase during pod formation to pod filling suggests that the seeds are the primary site of GCA accumulation. During the pod filling stage, the VS plants contained about 10% of the GCA content of 9.6–12.9 g kg⁻¹ DM reported for VS seeds (Aulrich and Böhm 2024). This seems plausible, given that the pods account for approximately 5.4%–7.2% of the total biomass, calculated by the yields of plant parts described by Rebolé et al. (2004). This percentage may vary depending on the variety and growing conditions. Ensiling significantly reduced the GCA content of VS plants to trace levels.

BCA was only detected during the pod filling stage of VS, and its contents were lower than those of GCA. The maximum whole-plant BCA content of VS at the pod filling stage was comparable to the minimum BCA content found in seeds (0.03–0.06 g kg⁻¹ DM) by Baldinger et al. (2022). This is a high BCA content compared to the seeds, considering the concentration dilution that occurs when sampling the whole plant. In contrast to Baldinger et al. (2022), ensiling did not result in an increase in BCA in this study, suggesting that GCA was not degraded into BCA. However, due to the low GCA and BCA contents, no definitive conclusion can be drawn. Ensiling significantly reduced the BCA content of VS plants.

Vicine was present in VS from the pod formation stage and in VP at the pod filling stage, indicating its production during seed formation. The vicine content in VS forage at the pod filling stage was four to six times lower than the seed vicine contents of 6.7–8.2 g kg⁻¹ DM measured by Baldinger et al. (2022) in the seeds. Ensiling was highly effective in reducing vicine, with degradation rates ranging from 99.9% to 100%. In this study, higher degradation rates than in the study of Baldinger et al. (2022) were achieved. While Baldinger et al. (2022) used only lactic acid bacteria as an additive, in the present

study, formic acid was added additionally. The high degradation rates may be attributed to the lower pH and lower initial vicine content, as well as to the different matrix. Acid hydrolysis is known to break down secondary metabolites, as confirmed by Aulrich and Böhm (2024) and Enneking and Wink (2000).

The growth stage, species and variety of vetch affect toxicity. However, ensiling significantly reduces the levels of GCA, BCA and vicine.

5 | Conclusion

Forages of different vetch species and varieties can be ensiled using the additive combination of 10L formic acid and 3.6×10^{11} CFU *L. plantarum* per ton. This additive combination achieves low AA and riboflavin losses and high degradation rates of SPM. However, the concentration of added formic acid and the additional effect of lactic acid bacteria need further evaluation, particularly, in relation to harvest stage and vetch species, which highly affect the composition of the plants. Overall, ensiling can preserve riboflavin and AA while degrading SPM problematic for animal nutrition, such as BCA, GCA and vicine. The result can be a safe, riboflavin- and lysine-rich roughage with high CP content.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All relevant data were included in this study. Further data analyses and publication will be carried out with regard to fresh material and cultivation as part of a 3-year trial. The corresponding author can be contacted for further explanations.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** gfs70025-sup-0001-Supinfo.docx.