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ABSTRACT

In organic pig production systems, one of the main challenges is to meet the demand for resources rich in protein. Among the resources available, temperate green plants, such as forage legumes, are potential sources of energy and protein. The aim of the study was to determine the nutritional value of silages (**S**) from the whole plant of lucerne (**L**) and red clover (**R**) and protein pastes (**PPs**) obtained from L and R leaves. In a first trial, 30 pigs were used in a factorial design to determine the total tract digestibility (**TTD**) of dietary nutrients and energy in five dietary treatments. The control group was fed a control diet (**C1**). The lucerne silage (**LS)** and red clover silage (**RS)** groups were fed a 78%:22% mixture (on a DM basis) of the C1 diet and LS or RS. The lucerne protein paste (**LPP)** and the red clover protein paste (**RPP)** groups were fed an 81%:19% mixture (on a DM basis) of the C1 diet and LPP or RPP. In the second trial, five pigs were used in a 5 × 5 Latin square design to evaluate the standardised ileal digestibility (**SID**) of amino acids (**AAs**) in the four legume products. The control diet (**C2**) was formulated with casein as the sole protein source. The LS and RS groups were fed an 85%:15% mixture (on a DM basis) of the C2 diet and LS or RS. The LPP and RPP groups were fed an 80%:20% mixture (on a DM basis) of the C2 diet and LPP or RPP. Regardless of the plant species, silages obtained from L and R leaves contained less AA and more fibre than protein pastes. While the fresh forages contained the same percentage of protein N in total N (63.6%), lucerne lost more protein N during ensiling than red clover (−75.5 vs −33.8%). The calculated TTD coefficient of energy was higher in silages than in protein pastes and lower in R than in L products (72.8, 71.5, 67.7, and 61.3 for LS, RS, LPP and RPP, respectively). The SID of total essential AA was higher in LPP than in RPP (87.2 vs 79.2%) whereas it was lower in LS than in RS (33.2 vs 56.8%). The lower SID values in silages were explained by the protein degradation during the ensiling process and a high proportion of AA linked to the NDF fraction. The results of the present study show that protein pastes obtained from lucerne and red clover are valuable protein sources for pig. In contrast, legume silages have to be considered as an energy source rather than a protein source.

Implications

The present study shows that protein concentrates obtained from fractionation of legumes forage with a screw press are good protein sources for swine. Legumes silages should be considered as an energy source rather than a protein source for pig feeding. The very low concentration of digestible amino acids in legume silage is related to the postharvest proteolysis of native proteins during ensiling proteolysis and the reduced amino acids digestibility coefficients due to high fibre content. Approaches for limiting proteolysis have to be implemented with the aim to improve the N use efficiency especially in lucerne silage.

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Introduction

Novel feed resources are explored to secure the future demand for food and protein. In this general context, novel protein-rich resources are required to feed livestock, especially monogastric animals. The difficulty in providing locally sourced protein is a major limiting factor for developing organic pig production. Among the resources available, temperate green plants, such as forage legumes, are potential sources of energy and protein for monogastric animals (Kambashi et al., 2014). They can be grown widely and improve the sustainability of cropping systems in rotation with cereals, especially in organic production systems. They also have a high protein yield with a balanced profile of amino acids (**AAs**) (Stødkilde et al., 2019). However, due to the high crude fibre (**CF**) content of the whole plant, these forage legumes are not suitable for monogastric animals (Kass et al., 1980). As leaves contain much more protein and less CF than stems, separating leaves at harvest is an option to improve the nutritional value of legume forages for pigs. To maintain their original nutritional quality for a long duration and to ensure that they remain easily available for feeding animals, leaves must be preserved by dehydration or ensiling. The nutritional value of legume forages composed of leaf meal for pigs has been described extensively in the literature (Andersson and Lindberg, 1997a; Reverter et al., 1999; Renaudeau et al., 2019). However, few studies have evaluated the energy digestibility in silages produced from the leafy part of legumes (Presto Åkerfeldt et al., 2018). In addition, the ileal AA digestible contents in legume silage have been not evaluated yet. A second method for using legume forages to feed pigs is to physically separate, by squeezing, the protein from the fibre to produce protein concentrates (Myer et al., 1975). These protein-rich fractions' DM generally contains 50–60% CP and an AA composition similar to that of soya bean protein (Santamaría-Fernández and Lübeck, 2020). Nutritional studies that estimate the energy and AA digestibility of green protein concentrates for pigs are scarce and have focused mainly on lucerne, and little is published about other commonly produced legume forages such as red clover (Stødkilde et al., 2019). The hypothesis to be tested in the present study was whether the botanical origin (lucerne vs red clover) of legume forage could influence the nutritional value of silages obtained from the leafy part of the plant or protein pastes obtained after squeezing the entire plant.

Material and methods

The experiment was performed at the experimental facilities of the Unité Expérimentale Physiologie et Phénotypage des Porcs in Saint-Gilles, France (https://doi.org/10.15454/1.5573932732039927E12), from October 2019 to March 2020.

Plants and processing

Lucerne (*Medicago sativa* L. "Creno") and red clover (*Trifolium pratense* L. "Suez") were grown on the experimental farm at Aarhus University, Foulum, Denmark. Lucerne was sown as pure stands in 2017 and red clover in 2018. The seeding rate was 33 kg/ha and 6 kg/ha for lucerne and red clover, respectively. The plants and leaves were harvested at a plant height of 55 and 35 cm for lucerne and red clover, respectively, and at a stubble height of 7–10 cm in May 2019 (lucerne) or August 2019 (red clover). The development stage at harvest was determined using the system proposed by Skinner and Moore (2007). The average maturity stage at harvest was index 3/4 for lucerne and index 6 for red clover. In the harvest year, both fields were fertilised with 230 kg/ha potassium chloride (K-50). Leaves were ensiled with crushed organic barley grain (200 kg per 1 000 kg leaves) but without additives in 200 L polyethylene drums. Based on a mini-silo laboratory experiment performed prior to the present study (Bani et al; personal communication), we waited three months before feeding lucerne and red clover silage (**LS** and **RS**, respectively) to the animals. Juices were extracted from whole plants using a screw press. Juices were precipitated via coagulation in a heat exchanger at 80 °C for 90–180 s (Corona et al., 2018). The resulting lucerne and red clover protein pastes (**LPP** and **RPP**, respectively) were subsequently frozen and thawed just before feeding them to the animals.

Experimental design, animal management and feeding

The study was divided into two digestibility trials. In trial 1, six blocks of five Pietrain × (Large White × Landrace) littermate barrows with an initial mean (±standard deviation) BW of 67.7 (±1.2) kg were used in two successive replicates to measure the TTD of LS, RS, LPP and RPP. Pigs were fed one of five dietary treatments (Table 1). The control group was fed a control diet (**C1**), based on wheat and soya bean meal, that contained 190 g/kg DM of CP and 15.3 MJ/kg DM of metabolisable energy (**ME**). The LS and RS groups were fed a 78%:22% mixture (on a DM basis) of the C1 diet and LS or RS. The LPP and the RPP groups were fed an 81%:19% mixture (on a DM basis) of the C1 diet and LPP or RPP. Regardless of the experimental group, ca. 23 g DM/kg of BW was fed daily. This slight feeding restriction homogenised feed intake among pigs and limited feed refusal and spillage, which avoided subsequent errors in digestibility measurements. Feed was provided as a fresh mixture of mash feed with water (1:2) and with or without LS, RS, LPP or RPP in two daily meals. Pigs had free access to water. The total duration of the trial was 21 d. During a 14-d period of adaptation to the diet, pigs were first housed in 1 × 1.2 m individual pens for 10 d and then in metabolic cages (0.6 × 1.2 m) for 4 d. All faeces and urine were collected for 7 consecutive d. The room temperature was held constant at 24 °C.

In trial 2, five Pietrain × (Large White × Landrace) barrows with an initial mean BW of 45.9 (±4.5) kg at the beginning of the exper-

Table 1
Ingredients and chemical composition of the experimental diets fed to the pigs (as fed).[1]

	Trial 1	Trial 2	
Diet	C1	C2	PF
Ingredient composition, kg/T			
Wheat	780.0		
Soybean meal	183.0		
Corn starch		676.5	829.6
Casein		153.0	0.0
Purified cellulose		40.0	40.0
Sugar		40.0	40.0
Soya bean oil		20.0	20.0
Others	22.0[2]	70.4[3]	70.4[3]
Chemical composition			
DM, %	87.7	89.2	90.0
Ash, % DM	6.40	5.90	5.41
Nitrogen, % DM	3.04	2.40	0.08
Starch, % DM			79.2
Ether extract, % DM	1.60	1.00	0.83
Crude fibre, % DM	2.68	0.90	2.17
NDF, % DM	13.1	5.4	3.16
ADF, % DM	2.87	0.6	2.22
ADL, % DM	0.59	0.0	0.66
Gross energy, MJ/kg DM	17.87	17.35	16.38

[1] C: control diet, PF: protein-free diet.
[2] Dicalcium phosphate (12 kg/T), calcium carbonate (15 kg/T), salt (5 kg/T) and premix (5 kg/T).
[3] Monocalcium phosphate (25.0 kg/T), sodium bicarbonate (25.0 kg/T), calcium carbonate (5 kg/T), potassium chloride (6 kg/T), magnesium chloride (3.6 kg/T), vitamins (0.8 kg/T), and premix (5 kg/T).

iment were used to measure the AID of nutrients from LS, RS, LPP and RPP. Pigs were prepared with an end-to-end ileorectal, antevalvular anastomosis as described by Laplace et al. (1989). Five dietary treatments were tested in a 5 × 5 Latin square design. The control diet (**C2**) was formulated with corn starch as an energy source and casein as the sole protein source (Table 1). The LS and RS groups were fed an 85%:15% mixture (on a DM basis) of the C2 diet and LS or RS. The LPP and RPP groups were fed an 80%:20% mixture (on a DM basis) of the C2 diet and LPP or RPP. At the end of the trial, all pigs were fed a protein-free diet during a 6th collection period. Feed was provided as a fresh mixture of mash feed with water (1:2) and with or without LS, RS, LPP or RPP in two daily meals. Feed was usually consumed quickly after the meal was distributed. Pigs were housed in metabolic pens for six consecutive periods of 7 d. Within each period, following a 4-d adaptation period, ileal digesta were collected for three consecutive days twice daily at 0800 and 1600 after feeding. In practice, all ileal digesta were recovered in 500 mL of 1 N H_2SO_4 in order to stop bacterial growth during their residence time (on average from 8 to 14 h) in each individual collection bin.

Pigs were weighed at the beginning and end of faeces collection in trial 1 and the beginning of the adaptation period and end of ileal digesta collection in trial 2. For each collection period, samples of C diets, silages and protein pastes were collected and analysed for DM content. For each trial, the samples were pooled per diet or raw material for subsequent chemical analysis. If present, feed refusals were collected daily and measured for DM content. In both trials, faecal and ileal digesta were collected daily, stored at 4 °C and weighed, homogenised and subsampled at the end of the collection period. These digesta samples as well as those of silages and pastes were freeze-dried for subsequent chemical analysis. Urine was also collected daily, and daily aliquots were pooled for each animal.

Sample analysis

For diet and raw material samples, AOAC (2000) methods were used to measure moisture, ash, nitrogen (**N**) (Dumas method; Leco 3000, St. Joseph, MI, USA), Weende CF and crude fat (extracted with petroleum ether; Soxtec Avanti2050; Foss, Höganäs, Sweden). Gross energy (**GE**) content was measured using an adiabatic bomb calorimeter (IKA C5000, Staufen, Germany). Cell wall fractions (NDF, ADF and ADL) were determined according to the methods of Van Soest and Wine (1967) using a sequential procedure with prior amylolysis (Termamyl® 120L) and sodium sulphite. Starch content was measured using the Ewers polarimetric method. The N and AA contents of the NDF residue were measured by successive analyses of NDF, N and AA. AAs were determined using high-performance liquid chromatography (HPLC) after hydrolysis with 6 N hydrochloric acid at 110 °C for 23 h. Methionine and cysteine underwent performic oxidation before hydrolysis. For tryptophan analysis, samples were hydrolysed with barite at 110 °C for 16 h. The methods used to analyse AAs were similar to those of Cozannet et al. (2010), who provide additional details. In trial 1, faecal samples were analysed for DM, ash, GE and N. In trial 2, ileal digesta were analysed for DM, ash, GE, N and AA. Additional analyses were conducted on silages and protein pastes, only. Condensed tannins were analysed using a method derived from Broadhurst and Jones (1978), polyphenol oxidase activity was analysed using a method derived from Lee et al. (2006), and isoflavonoid and sapogenin profiles were analysed via ultra-HPLC with internal methods developed by an external laboratory (BioGEVES, Surgères, France) derived from Oleszek and Stochmal (2002). To evaluate the quality of the silages, additional analyses (i.e. ammonia and soluble N, lactic acid and volatile fatty acids) were performed by another external laboratory (LABOCEA, Ploufragan, France).

Calculations and statistical analysis

In trial 1, dietary TTD of DM, organic matter (**OM**), N and energy were calculated for each pig from the chemical composition of the diets and faeces. The UNIVARIATE procedure of SAS was used to determine if there were any outliers. Data were subjected to variance analysis using the GLM procedure of SAS statistical software with a model that included the effects of diet, replicate and their interaction. In trial 2, AID of DM, OM, N, energy and AA were calculated for each pig and each period from N and AA contents in the diets and in ileal digesta. To calculate standardised ileal digestibility (**SID**), the apparent ileal digestibility (**AID**) was corrected for basal endogenous AA losses collected during the 6th period, which we assumed were related directly to DM intake (Mariscal-Landín et al., 2002). Data were subjected to variance analysis using the GLM procedure of SAS with a model that included the effects of diet, animal and period. In trials 1 and 2, TTD, AID and SID of the components of LS, RS, LPP and RPP were calculated from differences in digestibility coefficients of the C diets, the experimental diets and the percentages of components in test ingredients in the experimental diets.

Results

Chemical composition of legume silages and protein pastes

In trial 1, the mean DM contents of silages and protein pastes fed to animals were 27.3, 27.4, 30.2 and 28.0% for LS, RS, LPP and RPP, respectively. The corresponding values for trial 2 were 24.4, 27.5, 28.0 and 27.8%, respectively. On a DM basis, lucerne contained more ash than red clover in both silage and protein paste forms (Table 2). Regardless of the plant species, silages contained less N and ether extract and more fibre and starch than protein pastes (Table 2). The percentage of protein N in total N was higher in protein pastes than in silage. While the fresh forages contained the same percentage of protein N in total N (63.6%), lucerne lost more protein N during ensiling than red clover (Table 2; Fig. 1). The end products ammonia and soluble N are traditionally measured to determine the degree of proteolysis during ensiling. Both parameters were significantly higher in LS than in RS (11.0 vs 6.8 and 70.2 vs 33.2% for ammonia and soluble N, respectively). On a DM basis, RPP contained more ADL (4.4 vs 2.4%), OM (93.9 vs 84.1%) and GE (24.6 vs 22.2 MJ/kg) than LPP. The difference in AA content among the silages and pastes was related to the difference in total N contents and to their percentage of protein N content. The sum of essential AA contents was 4.0, 7.3, 27.4 and 27.8 g/100 g DM in LS, RS, LPP and RPP, respectively. The total N and AA contents associated with NDF from silages and protein pastes and their contribution to the total AA content in these products varied (Table 3). The contents of N and most of the AA were higher in NDF from silages than those from protein pastes. In silages, percentages of protein N associated with NDF in total N were higher in RS than in LS. In RS, percentages ranged from 33.9% (arginine) to 23.6% (methionine) for the essential AA. In LS, percentages among AA varied more, with the highest values for threonine (46.1%), lysine (45.2%), arginine (44.3%) and histidine (39.6%), and the lowest values for isoleucine (12.1%), tryptophan (12.5%), valine (12.7%), methionine (13.1%) and leucine (13.3%). These percentages ranged from 16.2% (histidine) to 21.5% (methionine) in LPP and from 18.6% (tryptophan) to 24.0% (methionine) in RPP. Contents of some secondary plant compounds in fresh whole plants and leaves, silages and protein pastes are pre-

Table 2
Analysed composition of the silages and protein pastes fed to the pigs[1] (% DM).

	Silages		Protein pastes	
Item	Lucerne	Red clover	Lucerne	Red clover
Ash	7.6	5.5	15.9	6.1
Organic matter	92.4	94.5	84.1	93.9
Total Nitrogen (Nt)	4.2	3.1	8.6	8.9
Protein N, %Nt	24.5	66.2	82.6	81.8
Ammonia N, %Nt	11.0	6.8	NA	NA
Soluble N, %Nt	70.2	33.2	NA	NA
NDF	25.8	29.3	15.8	15.6
ADF	10.4	11.5	4.5	6.7
ADL	2.1	2.2	2.4	4.4
Crude fibre	10.7	10.4	0.7	1.2
Ether extract	4.4	3.8	10.2	11.1
Starch	25.7	32.7	0.9	0.3
Gross energy, MJ/kg DM	20.5	19.2	22.2	24.6
Essential AA				
Arginine	0.15	0.62	3.28	3.33
Histidine	0.09	0.36	1.24	1.27
Isoleucine	0.61	0.75	2.90	2.84
Leucine	0.90	1.40	4.98	5.11
Lysine	0.21	0.86	3.57	3.48
Methionine	0.28	0.37	1.07	1.00
Phenylalanine	0.57	0.88	3.25	3.33
Threonine	0.14	0.72	2.57	2.71
Tryptophan	0.25	0.26	1.18	1.25
Valine	0.76	1.05	3.39	3.51
∑Essential AA	3.96	7.27	27.4	27.8
Non-essential AA				
Alanine	1.40	0.98	3.23	3.26
Aspartic acid	0.31	1.59	5.17	5.32
Cysteine	0.11	0.20	0.39	0.32
Glutamic acid	0.74	2.32	5.78	6.05
Glycine	0.62	0.86	2.79	2.90
Proline	0.55	1.18	2.40	2.57
Serine	0.17	0.70	2.33	2.43
Tyrosine	0.15	0.57	2.37	2.6
∑Non-essential AA	4.05	8.40	24.5	25.5

Abbreviation: AAs = amino acids.
[1] Mean DM contents of lucerne silage, red clover silage, lucerne protein paste and red clover protein paste were 27.5, 27.4, 30.2 and 28.0%, respectively, in trial 1 and 24.4, 27.5, 28.0, 27.8%, respectively, in trial 2. NA for not analysed.

sented in Table 4. Whole plants and leaves of red clover contained more condensed tannins than those of lucerne, but this difference was not observed in their silage or protein paste forms. Regarding the isoflavonoid contents, red clover contained much more biochanine A, formononetin and genistein than lucerne. Highest concentrations of these isoflavonoids were found in fresh leave and protein paste. In contrast, lucerne contained more luteolin, tricin and apigenin in the whole plant, leaves and silages. Logically, lucerne products contained more saponins (especially medicagenic acid, bayogenin, and soyasapogenol B) than red clover products. Based on our analyses, saponin concentrations were generally higher in LPP than in LS. Polyphenol oxidase activity was higher in red clover than in lucerne, especially in the leaves.

Growth performance

All animals remained healthy throughout both trials. None of the pigs had to be removed from the study, and no spillage or refusals were observed. Pig performances during the 10-d experimental period of trial 1 varied (Table 5). On a DM basis, the C1 + LS and C1 + RS diets contained the same percentage of silage (22.3%), while the C1 + LPP diet contained slightly less protein paste than the C1 + RPP diet (17.4 vs 19.9%, respectively). Average daily weight gain was highest in pigs fed pastes (average of LPP and RPP = 874 g/d) and lowest in pigs fed C + LS (571 g/d), while pigs fed C and C + RS had intermediate values (671 and 614 g/d, respectively). As daily DM intake was kept the same for all treatments, results for feed conversion ratio were consistent with those obtained for average daily gain (Table 5).

Total tract and ileal digestibility of diets and legume forages

Inclusion of legume products decreased DM, OM, N and energy apparent TTD ($P < 0.05$; Table 5), which had direct consequences on dietary values and the N balance of the pigs. Whatever the nutrient, lowest TTD were observed for the C + RPP diet. Similarly, AID of OM, N, energy and AA also decreased when silages or protein pastes were included in the diets (Table 6). On average, the AID of essential AA was 6.2 percentage points lower in diets with 15% of silage compared to the C diet. Compared to the AID of essential AA for the C diet, those for the C + LPP and C + RPP diets were 4.9 and 8.8% lower, respectively, when 20% paste was added to the C diet. Losses of endogenous AA, except for glycine and proline, varied little among animals (Table 7). Under our experimental conditions, essential AA represented only 35% of total endogenous losses.

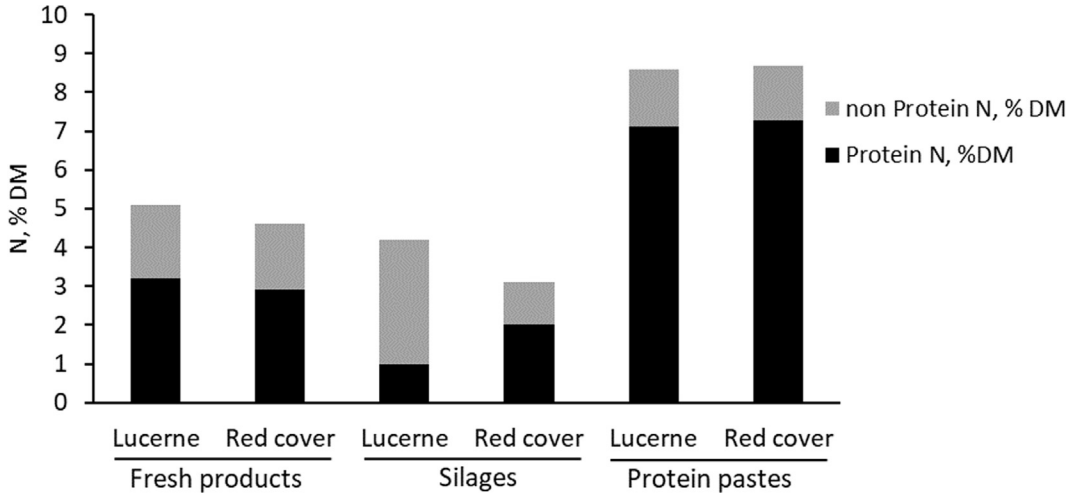

Fig. 1. Distribution of protein and non-protein nitrogen (N) in fresh products, silages and protein pastes in the pig study.

Table 3
Contents of protein N and AA associated with the neutral detergent fibre (% DM) and their contribution to the protein N and AA contents in silages and protein pastes fed to the pigs.

Item	Silages				Protein pastes			
	Lucerne		Red clover		Lucerne		Red clover	
	Cont.	Contr.	Cont.	Contr.	Cont.	Contr.	Cont.	Contr.
Total Nitrogen (Nt)	0.36	8.6	0.79	25.5	1.44	16.7	1.68	18.9
Protein N	0.19	19.5	0.52	25.6	1.20	17.3	1.39	19.7
Amino acid								
Essential AA								
Arginine	0.07	44.3	0.21	33.9	0.59	17.9	0.68	20.4
Histidine	0.04	39.6	0.09	25.9	0.20	16.2	0.24	19.1
Isoleucine	0.07	12.1	0.20	26.6	0.48	16.4	0.55	19.2
Leucine	0.12	13.3	0.34	24.5	0.84	16.9	0.97	19.0
Lysine	0.10	45.3	0.22	25.5	0.60	16.9	0.66	19.1
Methionine	0.04	13.1	0.09	23.6	0.23	21.5	0.24	24.0
Phenylalanine	0.09	15.8	0.24	27.4	0.56	17.2	0.64	19.2
Threonine	0.06	46.1	0.18	24.3	0.45	17.7	0.52	19.2
Tryptophan	0.03	12.5	0.07	25.8	0.20	16.9	0.23	18.6
Valine	0.10	12.7	0.25	24.0	0.57	16.9	0.72	20.6
∑Essential AA	0.71	17.9	1.89	26.0	4.72	17.2	5.46	19.6
Non-essential AA								
Alanine	0.08	5.4	0.20	20.6	0.53	16.4	0.61	18.8
Aspartic acid	0.12	40.2	0.32	20.4	0.88	16.9	1.06	19.9
Cysteine	0.03	30.1	0.06	28.6	0.09	24.1	0.09	26.9
Glutamic acid	0.14	18.5	0.56	24.3	0.97	16.7	1.20	19.9
Glycine	0.10	16.7	0.22	26.1	0.49	17.5	0.57	19.8
Proline	0.09	16.0	0.28	23.4	0.42	17.6	0.50	19.4
Serine	0.08	49.6	0.19	27.1	0.42	18.0	0.45	18.4
Tyrosine	0.08	53.8	0.18	31.9	0.42	17.9	0.52	20.1
∑Non-essential AA	0.73	17.7	2.02	24.0	4.22	17.2	5.01	19.6

Abbreviations: N = nitrogen; AAs = amino acids; Cont. = contents; Contr. = contribution.

Table 4
Tannin, flavonoid and sapogenin concentrations and PPO activity in whole plants, leaves, silages and protein pastes obtained from lucerne and red clover fed to the pigs.[1]

Item	Lucerne				Red Clover			
	Whole plant	Leaves	Silage	Protein paste	Whole plant	Leaves	Silage	Protein paste
Condensed tannins, % catechin equivalent	0.38	0.34	0.25	0.22	0.61	0.69	0.20	0.27
Isoflavonoids, mg/g DM								
Biochanine A	UloQ	ULoQ	ULoQ	ULoQ	3.16	5.56	2.97	6.63
Formononetin	UloQ	0.03	0.03	UloQ	3.32	4.45	2.51	8.63
Genistein	UloQ	ULoQ	ULoQ	ULoQ	0.20	0.23	0.15	0.34
Coumestrol	UloQ	0.06	0.03	0.02	ULoQ	0.02	0.06	0.03
Luteolin	0.11	0.06	0.17	ULoQ	ULoQ	ULoQ	ULoQ	ULoQ
Tricin	1.25	0.88	1.85	0.06	0.13	0.15	0.09	0.07
Apigenin	0.08	0.11	0.62	0.04	0.01	0.01	ULoQ	0.02
Sapogenins, mg/g DM								
Medicagenic acid	0.93	1.00	1.18	1.70	0.13	0.20	0.13	0.20
Bayogenin	0.10	0.42	0.29	0.45	0.15	0.27	0.10	0.12
Soyasapogenol B	0.09	0.13	0.11	0.23	ULoQ	0.06	ULoQ	0.11
Oleanolic acid	0.16	0.12	ULoQ	ULoQ	0.30	0.27	ULoQ	0.15
PPO, A/min/g DM	0.46	0.36	0.0	0.0	0.53	0.54	0.0	0.0

Abbreviations: PPO = polyphenol oxidase; ULoQ = below the limit of quantification.

The calculated TTD coefficients of OM, N and GE and the SID of N and AA of silage and pastes are presented in Table 8. The TTD of OM and energy were slightly lower in RS than in LS. This difference was larger for protein pastes. The TTD of energy was lower for RPP (61.3%) than for LPP (67.7%). Consequently, the DE of lucerne products was slightly higher than that of red clover products. The distributions of DE between the small and large intestines were 76%:24 and 72%:28% in LS and RS, respectively. Most of the energy in the protein pastes (mean of 98%) was digested in the small intestine (Fig. 2). The SID of AA and N were calculated after correcting for the basal endogenous contribution of AA at the terminal ileum based on the protein-free diet. The SID of essential AA was significantly lower in LS than in RS (33.2 vs 56.8%, respectively), due to the negative SID of arginine, histidine, lysine and threonine in LS. The same trend was observed for non-essential AA. In protein pastes, the SID of AA were higher in LPP than in RPP. The difference in SID of the essential AA was 8 percentage points.

Discussion

Influence of plant species and processing method on chemical composition

Due to legumes' high fibre content, which decreases protein digestibility, removing of their fibre and extracting their protein effectively increase their nutritional value for monogastric animals (Stødkilde et al., 2019). In the present study, we used heat coagu-

Table 5
Growth performance, nitrogen balance and total tract digestibility coefficients in experimental diets fed to the pigs (trial 1).[1]

Item	C1	C1 + LS	C1 + RS	C1 + LPP	C1 + RPP	SEM	Statistics[2]
No. of pigs	6	6	6	6	6		
Live BW, kg							
Initial	67.4	69.6	67.2	68.0	66.4	7.3	
Final	72.1	73.6	71.5	74.2	72.4	7.2	
Average BW gain, g/d	671^{b}	571^{c}	614^{bc}	886^{a}	862^{a}	116	D**, R*
Feed allowance (as fed), g/d							
Control	1 800	1 430	1 430	1 430	1 430	–	
Other	0	1 310	1 305	870	1 075	–	
Total	1 800	2 740	2 735	2 300	2 505	–	
Feed intake, g DM/d	1 565	1 601	1 602	1 503	1 553	–	
Incorporation of legume products, % of DM	0	22.3	22.3	17.4	19.9	–	
Feed conversion ratio, g DM/g	2.49^{b}	3.12^{a}	2.67^{ab}	1.73^{c}	1.82^{c}	0.62	D**, R*
Faeces DM, %	24.1^{b}	16.7^{d}	19.8^{c}	28.7^{a}	25.2^{b}	2.4	D**, R**
Digestibility coefficient, %							
DM	88.0^{a}	85.2^{b}	85.1^{b}	82.9^{c}	82.6^{c}	0.6	D**, R**
Organic matter	90.1^{a}	86.9^{b}	86.6^{b}	86.5^{b}	84.5^{c}	0.5	D**, R**
Nitrogen	86.8^{a}	82.8^{b}	81.6^{b}	83.5^{b}	78.8^{c}	1.5	D**, R*
Energy	87.8^{a}	84.1^{b}	83.4^{b}	82.5^{b}	81.2^{c}	0.6	D**, R*
DE, MJ/kg DM	15.70^{a}	15.51^{b}	15.36^{c}	15.55^{b}	15.48^{bc}	0.11	D**, R**
ME, MJ/kg DM	15.29^{a}	15.06^{b}	14.99^{b}	15.11^{b}	15.03^{b}	0.12	D**, R**
Nitrogen balance, % intake							
Faecal losses	13.2^{c}	17.2^{b}	18.4^{b}	16.5^{b}	21.2^{a}	1.6	D**, R*
Urine losses	39.7^{ab}	41.7^{a}	36.1^{bc}	32.9^{c}	33.3^{c}	4.1	D**
Retained	47.1^{a}	41.0^{c}	45.5^{ab}	50.6^{a}	45.5^{ab}	4.1	D*

Abbreviations: C = control diet; LS = lucerne silage; RS = red clover silage; LPP = lucerne protein paste; RPP = red clover protein paste; DE = digestible energy; ME = metabolisable energy.
[a, b, c, d]Means within a row without a common superscript letter differ ($P < 0.05$).
[1] On a DM basis, LS, RS, LPP, and RPP were incorporated at 22.3%, 22.3%, 17.4 and 19.9% in C + LS, C + RS, C + LPP and C + RPP, respectively.
[2] From a GLM model of variance including the effects of diet (D), replicate (R) and their interaction.
* $P < 0.05$.
** $P < 0.01$.

Table 6
Apparent ileal digestibility coefficients for feed components in experimental diets fed to the pigs (trial 2).[1]

Item	C2	C2 + LS	C2 + RS	C2 + LPP	C2 + RPP	SEM	Statistics[2]
No. of pigs	5	5	5	5	5		
Mean live BW, kg	56.1^{a}	54.0^{a}	54.7^{a}	55.2^{a}	53.4^{a}	1.1	
Feed intake, g DM/d	1 083	1 080	1 087	1 080	1 080	–	
Apparent ileal digestibility coefficient, %							
Organic matter	92.0^{a}	86.3^{bc}	86.2^{bc}	86.6^{b}	84.7^{c}	0.8	D**, P**, A*
Nitrogen	91.8^{a}	84.2^{bc}	82.9^{bc}	86.1^{b}	80.8^{c}	2.1	D**
Energy	92.4^{a}	86.0^{b}	85.6^{b}	85.9^{b}	83.7^{c}	1.1	D**, A*
Essential AA							
Arginine	94.5^{a}	86.2^{b}	86.1^{b}	92.0^{a}	87.3^{b}	1.8	D**
Histidine	97.4^{a}	92.1^{b}	91.3^{b}	92.1^{b}	87.6^{c}	1.3	D**, P*
Isoleucine	94.1^{a}	87.7^{bc}	87.4^{bc}	89.7^{b}	84.5^{c}	1.8	D**, A*
Leucine	97.3^{a}	92.6^{b}	91.9^{bc}	92.4^{bc}	89.5^{c}	1.3	D*
Lysine	97.6^{a}	93.4^{b}	92.8^{b}	93.4^{ab}	89.7^{c}	1.1	D**
Methionine	95.2^{a}	89.5^{b}	88.4^{b}	90.5^{b}	87.3^{b}	2.5	D**, A*
Phenylalanine	98.1^{a}	93.1^{b}	92.7^{b}	92.5^{b}	88.7^{c}	1.4	D**, P*
Threonine	94.4^{a}	84.0^{c}	84.4^{c}	89.2^{b}	84.8^{c}	1.1	D**, A*
Tryptophan	96.7^{a}	86.4^{b}	84.6^{b}	90.2^{b}	85.6^{b}	2.9	D**, P**, A*
Valine	95.9^{b}	89.9^{bc}	89.1^{bc}	90.6^{b}	87.0^{c}	1.3	D**
∑Essential AA	96.4^{a}	90.4^{bc}	89.9^{bc}	91.5^{b}	87.6^{c}	2.0	D**
Non-essential AA							
Alanine	92.8^{a}	84.7^{b}	82.6^{b}	88.1^{ab}	83.4^{b}	3.0	D**
Aspartic acid	94.5^{a}	86.7^{c}	87.3^{c}	90.0^{b}	84.9^{bc}	1.5	D**
Cysteine	72.8^{a}	43.8^{c}	48.8^{bc}	59.2^{b}	30.8^{d}	5.5	D**, P*, A*
Glutamic acid	95.7^{a}	91.4^{b}	91.2^{b}	91.6^{b}	88.9^{b}	1.2	D**, A**
Glycine	84.6^{a}	70.3^{b}	71.8^{b}	82.8^{a}	75.6^{b}	3.0	D**
Proline	95.5^{a}	92.4^{b}	90.9^{b}	92.1^{b}	89.8^{b}	1.3	D**, A**
Serine	90.5^{a}	80.1^{b}	80.6^{b}	84.8^{b}	81.1^{b}	2.4	D**, A**
Tyrosine	97.7^{a}	93.1^{b}	92.5^{b}	93.8^{b}	89.7^{c}	1.0	D**, P*
∑Non-essential AA	94.8^{a}	88.4^{bc}	88.1^{bc}	89.8^{b}	85.9^{c}	1.3	D**

Abbreviations: C = control diet; LS = lucerne silage; RS = red clover silage; LPP = lucerne protein paste; RPP = red clover protein paste; AAs = amino acids.
[a, b, c, d]Means within a row without a common superscript letter differ ($P < 0.05$).
[1] On a DM basis, LS, RS, LPP, and RPP were incorporated at 15.5%, 15.9%, 20.3 and 20.5% in C + LS, C + RS, C + LPP and C + RPP, respectively.
[2] From a GLM model of variance including the effects of diet (D), period (P) and animal (A).
* $P < 0.05$.
** $P < 0.01$.

Table 7
Basal endogenous AA losses in pigs used in the trial 2 (g/kg DM feed intake).

Item	Mean	SD
Essential AA		
Arginine	0.37	0.10
Histidine	0.13	0.01
Isoleucine	0.26	0.03
Leucine	0.43	0.05
Lysine	0.35	0.06
Methionine	0.11	0.05
Phenylalanine	0.25	0.03
Threonine	0.38	0.03
Tryptophan	0.13	0.01
Valine	0.37	0.03
Non-essential AA		
Alanine	0.34	0.08
Aspartic acid	0.63	0.08
Cysteine	0.20	0.01
Glutamic acid	0.73	0.08
Glycine	0.67	0.21
Proline	1.94	1.64
Serine	0.40	0.04
Tyrosine	0.24	0.03

Abbreviation: AAs = amino acids.

Table 8
Nutritional values of silages and protein pastes in growing pig (% DM).

	Silages		Protein pastes	
	Lucerne	Red clover	Lucerne	Red clover
Total tract digestibility coefficients, %				
Organic matter	76.5	74.5	67.9	60.8
Nitrogen	68.7	66.4	74.8	64.2
Energy	72.8	71.5	67.7	61.3
Energy values[1], MJ/kg DM				
DE	14.9	14.2	15.2	15.1
ME	14.3	13.8	14.4	14.5
Standardised Ileal digestibility, %				
Protein N	21.6	54.7	86.3	77.7
Non-protein N	82.7	59.7	83.3	64.5
Total N	68.5	56.4	85.7	75.3
Amino acids				
Essential AA				
Arginine	−51.2	54.3	91.5	84.0
Histidine	−32.4	56.3	85.9	75.9
Isoleucine	48.6	55.2	86.1	75.6
Leucine	54.3	64.8	87.4	81.5
Lysine	−45.3	55.0	87.8	78.5
Methionine	43.4	47.7	83.9	74.6
Phenylalanine	59.2	69.6	87.9	80.6
Threonine	−160.8	40.5	85.4	77.1
Tryptophan	59.0	53.8	88.3	81.8
Valine	46.2	54.8	84.9	77.3
∑Essential AA	33.2	56.8	87.2	79.2
Non-essential AA				
Alanine	72.3	59.9	86.5	79.1
Aspartic acid	−62.4	61.7	87.1	77.6
Cysteine	−49.1	13.4	54.0	42.4
Glutamic acid	−15.1	56.9	82.6	74.4
Glycine	41.8	54.9	84.1	74.0
Proline	46.3	60.8	85.3	77.6
Serine	−203.4	18.0	77.6	69.0
Tyrosine	−35.0	55.4	89.2	80.3
∑Non-essential AA	18.7	54.1	84.1	75.0

Abbreviations: AAs = amino acids; DE = digestible energy; ME = metabolisable energy.

lation to precipitate proteins from lucerne and red clover green juices. According to Damborg et al. (2020), this method can recover more than 60% of the total CP content in both forages. The main advantage of protein precipitation is that it reduces the amount of fibre-bound proteins, which are less digestible for pigs than soluble proteins. In the present study, NDF-bound N contributed 17 and 19% of the total N content of LPP and RPP, respectively. In theory, AA associated to NDF are only partially digested because the digestive enzymes have limited access to the cell wall components as well as the cell content enclosed by them (Schulze et al., 1994). The large contribution NDF-bound N to the total N is firstly explained mainly by the NDF content in LPP and RPP, which suggests that screw pressing does not retain all plant fibre in the pulp fraction and/or that filtration and decanting do not completely remove the remaining fibre. Alternatively, we cannot totally exclude that thermal coagulation has partially denatured the proteins contained in the juice, making them insoluble in the NDF solution with possible consequences on the NDF and NDF-bound N determination. As Damborg et al. (2020) highlighted, only a small amount of non-protein N (NPN) content remains after protein precipitation. In their study, the total AA content was ca. 82.0% of the total N content, which agrees with the percentages in the present study (82.6 and 81.8% in LPP and RPP, respectively). The total amount of AA and the profiles of individual AA were similar in LPP and RPP, mainly because the conserved enzymatic protein RuBisCO is the main N source in protein concentrates extracted from plant biomass (Collins, 1986). In addition, the total amount of AA (a mean of 530 g/kg DM) and the profiles of individual AA in protein pastes were similar to those reported for soybean meal. This confirms that protein pastes from legume forages are a valuable source of protein for pigs.

In the present study, no negative sensory defects, such as evidence of bad fermentation, heat or mould growth, were observed, either immediately or several days after opening the drums. In LS, NPN represented ca. 75% of the total nitrogen (vs. 44% in RS), which indicates extensive protein losses during ensiling of lucerne. This greater breakdown of protein in LS than in RS was confirmed by increased ammonia N and soluble N contents in LS. Protein in ensiled forage breaks down in two steps: proteolysis by plant proteases, followed by deamination of free AA into ammonia by microbial enzymes. Studies have attributed inhibited proteolysis to polyphenol oxidases (Mayer, 1986), that are naturally present and active in red clover leaves. Damaged cells release polyphenol oxidases, which catalyse the oxidation of endogenous o-diphenols to quinones in the presence of oxygen. The production of o-quinones in red clover inhibits postharvest proteolysis during ensiling (Schmitz et al., 2008). However, proteolysis also depends on pH, which influences the activity and stability of proteases, especially at the beginning of ensiling. Unfortunately, the pH drop occurring in LS and RS was not measured in our study. Lactic and acetic acids contribute the most to the decrease in pH during fermentation. In the present study, lactic and acetic acid contents were similar in both silages (2.7 vs 2.1 g/100 g fresh material for lactic acid and 2.4 vs 1.8 g/100 g fresh material for acetic acid in LS and RS, respectively; data not shown) and agreed with values previously recorded for lucerne leaf silage harvested on the first cut (Muck et al., 2010). These results suggest that the differing protein losses in LS and RS were related mainly to differences in polyphenol oxidase activity than to differences in the decrease in pH. In the present study, the ammonia content was 1.6 times as high in LS as in RS. Ammonia content can be used to estimate microbial activity in ensiled forage, mainly because deamination breaks proteolysis products (e.g., peptides and free AA) down into ammonia. In addition, butyric acid was detected only in LS (data not shown). Even though its content was relatively low (0.13%), butyric acid is found more often after clostridial fermentation (Li et al., 2018). As clostridia are highly proteolytic (Kung et al., 2018), the higher protein losses observed in LS could have been related in part to increased metabolic activity of clostridia. Due to this loss in protein during ensiling, the total content of essential

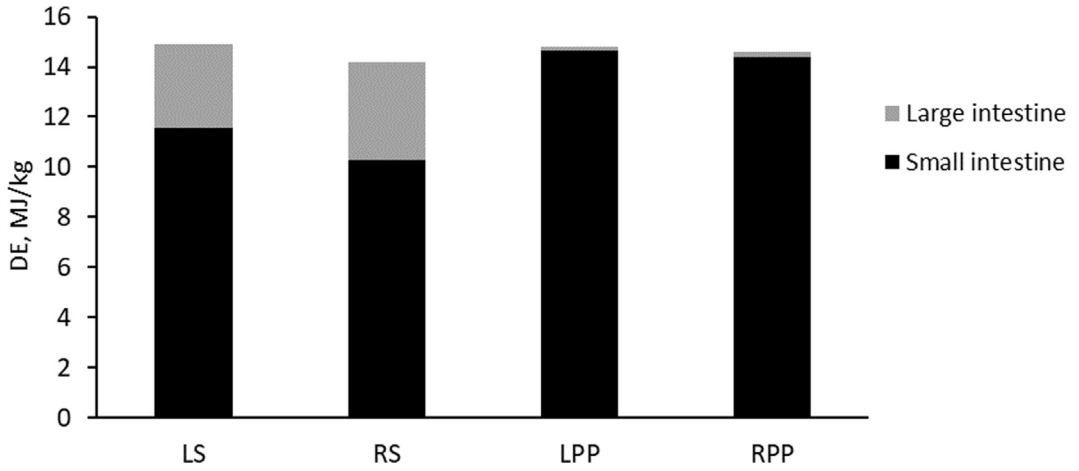

Fig. 2. Digestible energy (DE; MJ/kg DM) contents in legume forage silages and pastes coming from the small and large intestines in growing pigs. LS: lucerne silage, RS: red clover silage, LPP: lucerne protein paste, RPP: red clover protein paste.

AA was 45 percentage points lower in lucerne products than in red clover products. The decrease in AA content in LS was particularly large for arginine, histidine, lysine and threonine, but small for branched-chain AA and methionine. In addition, our results indicate that a large proportion of arginine, histidine, lysine and threonine were bound to NDF in LS. These results indicate that the high protein loss during lucerne ensiling reduced the quantity, quality and availability of dietary protein.

The feeding value of protein pastes or silages depends not only on the nature and on the concentration of dietary fibre but also on the presence of secondary compounds that can be considered as anti-nutritional factors for pig nutrition. In agreement with Butkuté et al. (2018), lucerne and red clover products used in the present study were differentiated mainly by their contents in isoflavones (fromononetin and biochanin A) and in saponins (medicagenic acid, bayogenin). Whatever the botanical origin of the plant, protein pastes contained much more isoflavones (red clover) or saponins (lucerne) than silages. Even though isoflavones fromononetin and biochanin A are primarily known as phytoestrogens, Li et al. (2020) indicated that these compounds also have antioxidant and anti-inflammatory properties and they would improve the absorption capacity of the small intestine in piglets. Many forage legumes grown in temperate area contain saponins with a high variety of biological effects with both positive and negative implications for animal production (Sen et al., 1998). One mechanism that usually explains the growth-depressing effects of saponins in monogastrics animals is the lowering of feed intake because of unpalatability (bitterness of saponins). In addition, lucerne saponin would lower the digestibility of nutrient in monogastrics animal by making indigestible complex with dietary fat and/or through an inhibition of digestive enzymes (Cheeke, 1971). As saponin contents increased in LPP and RPP, one can be suggested that the above-mentioned depressive effect in nutrient digestibility would be emphasised in protein concentrates.

Energy value of legume silages and protein pastes

In the present study, including 22% silage (on a DM basis) in experimental diets decreased the TTD of energy compared to that of the control diet. This decrease in nutrient and energy digestibility was expected based on the higher fibre contents in silages and the negative relationship between dietary NDF content and energy digestibility. To our knowledge, the literature contains few data on the energy value of forage silages for pigs. Jorgensen et al. (2012) estimated that the TTD of energy of clover silage was ca. 50% in growing pigs. A lower TTD (36%) was reported for whole plant clover-grass silage in pigs (Presto Åkerfeldt et al., 2018). In the present study, the TTD of energy coefficient of LS and RS was much higher (ca. 72%), which agrees with the 73% reported for ensiled lucerne leaves by Malmlof et al. (1990). Silages produced from the leaves of legume forages have higher TTD of energy because leaves contain less NDF than whole plants. However, adding barley to the fresh material to increase the DM of the mixture and to facilitate ensiling also probably increased the TTD of energy by increasing the starch content and diluting the cell wall fraction. More generally, the relatively large differences in the TTD of energy of silages among studies are likely related to differences in their harvesting conditions (e.g. stage, DM of the fresh material) and/or the method used to assess their nutritional value, and in particular their percentage inclusion in experimental diets. Due to the difference in energy digestibility, LS had slightly higher DE and ME than RS. Similar differences were reported between lucerne and red clover meals (Andersson and Lindberg, 1997a; 1997b). Based on previous studies (Shi and Noblet, 1993; Iyayi and Adeola, 2015), hindgut fermentation can supply 10–15% of DE to growing pigs fed a cereal-based diet. In the present study, the hindgut supplied 24 and 28% of DE from LS and RS, respectively. As fibrous ingredients are digested mainly by the hindgut, its large contribution to the total DE of silages is due to their higher fibre contents. This agrees with Lindberg and Cortova (1995), who calculated that each percentage increase in CF intake of lucerne leaf meal increased the contribution of hindgut to the digestibility of energy by 4%. The greater contribution of the hindgut to energy digestibility of RS than of LS was related directly to the former's higher fibre content.

Whatever the botanical origin of the plant, energy digestible coefficients were lower in protein pastes than in silages while NDF content was greater in silages. This result has first to be connected to the higher starch content in silages but is inconsistent with the higher ether extract content in protein pastes. However, according to the negative effect of saponins on fatty material digestibility and the higher saponin concentration in protein pastes, we can assume that reduced energy digestibility would be related to a reduced dietary fat absorption. In the present study, the energy digestibility of protein pastes from lucerne and red clover was lower (<68%) than that of traditional soya bean meal (>85%; (Sauvant et al., 2004)). Although both raw materials have rather similar NDF contents, it can be assumed that the physicochemical properties of their cell wall fibre differ greatly, especially the soluble and insoluble fibre contents. Insoluble fibre, which represents a large proportion of dietary fibre in legume forages (Chen

et al., 2014), has a resistance to microbial fermentation, which decrease energy digestibility (Wenk, 2001). This last point is consistent with the very small amount of energy digested in the large intestine (<2%). The TTD of energy of LPP in the present study (68%) generally agreed with those of Bourdon et al. (1980) (73%) and Callu et al. (2013) (65%). Due to LPP's lower GE, however, the DE of LPP was slightly lower than those in these two studies (15.0 vs 15.8 and 15.7 MJ/kg DM, respectively). The literature contains no data on the energy value of red clover protein contents. In the present study, the energy digestibility of RPP was 6 percentage points lower than that of LPP. This lower TTD of RPP could be related to its higher lignin content. As LPP had lower GE than RPP, its DE was similar to that of RPP (15.2 and 15.1 MJ/kg DM, respectively).

Protein value of legume silages and protein pastes

Based on literature data, silages from legume forage are generally considered a valuable source of protein for pigs (Presto Åkerfeldt et al., 2018). As mentioned before, ensiling broke down a significant part of the protein, especially in lucerne leaves, which produced a large amount of NPN. Our results indicate that a large amount of NPN is absorbed before the end of the ileum. In silages, the NPN fraction is a heterogeneous mixture of several N compounds in various proportions, which can include free AAs, peptides, amides, ammonia and unidentified NPN compounds (Givens and Rulquin, 2004). The way in which N is absorbed from the gut and the influence of this NPN supply on the N balance in pigs is debatable. Based on the increase in urine N excreted by pigs fed the LS diet, we hypothesise that the absorption of a large amount of NPN was not apparently used for protein deposition in swine. However, additional studies are needed to verify this hypothesis. For example, in certain situations, some of the urea flux can also be recycled in the small intestine and used by microflora to synthesise new AA (Fuller and Reeds, 1998).

The SID coefficients of AA in LS, estimated by the difference between the control and LS diets, were low (a mean of 22%). Due to the low AA content of LS, the small contribution of AA in LS (8%) to the total AA intake by the LS pigs might have resulted in unreliable estimates for the SID of AA. In addition, some AA (arginine, histidine, lysine, threonine, asparagine, cystein, serine, and tyrosine) had a negative SID. Some of the variation in AA digestibility is usually due to differences in the NDF content of feedstuffs. High fibre content decreases the AID of protein due to increased ileal losses of endogenous proteins (Mariscal-Landín et al., 2017). These losses are considered in part when ileal AA fluxes are corrected by non-specific basal endogenous losses estimated by feeding pigs a protein-free diet. However, protein and AAs associated with the NDF fraction are likely to have low digestibility in silage because digestive enzymes have limited access to cell wall components and cell contents (Bjergegaard et al., 1991). Due to the difference in proteolysis rate during ensiling, RS contained more AAs, and these AAs were more digestible than those in LS. This higher digestibility could also be due to a lower percentage of protein N and AA associated with NDF in the total CP and AA in RS. The SID of lysine in RS (expressed as g/16 g protein N) was 32% lower than that usually reported for soya bean meal. For the other AAs, their profiles in RS differed greatly from those of soya bean meal, with higher contents of branched-chain AAs (methionine, phenylalanine, threonine, aspartic acid and serine) and lower contents of histidine, arginine, tryptophan, alanine, cysteine, and tryptophan.

The nitrogen fraction of protein pastes from whole legume plant juices are much more digestible than silages, probably due to their lower fibre contents. In the present study, the total N SID of LPP and RPP (85.7 and 75.3%, respectively) were similar to those reported by Callu et al. (2013) for pigs (76.4% in lucerne protein concentrate) and Stødkilde et al. (2019) for rats (85.0 and 77.4% in lucerne and red clover protein concentrate, respectively). The latter authors already highlighted a significant effect of plant species on protein digestibility. Higher CF content and percentage of N associated with the NDF fraction could partly explain the lower N SID in RPP. In addition, DF from the RPP have a slightly higher water holding capacity (WHC) than those from LPP (2.7 vs 2.1 kg/kg DM; $P < 0.01$; results not showed). As reviewed by Souffant (Souffrant, 2001), WHC seems to be one of the most important factors influencing the ileal digestibility of nutrients and endogenous losses in pigs. Thus, the slight difference in WHC might partly explain the lower N digestibility reported for RPP. However, further works are needed to verify this hypothesis The SID of lysine in LPP and RPP were lower than that in soybean meal (87.8 and 78.5 vs 90.0%, respectively). This lower digestibility is probably related to the difference in CF contents between protein pastes and soya bean meal. However, as mentioned before, we cannot totally exclude that thermal coagulation has partially denatured the proteins with detrimental effects on AA digestibility. However, the amounts of SID lysine provided by LPP and RPP were higher than or similar to the mean value reported for soya bean meal (31.4 and 27.3 vs 28.4 g/kg DM, respectively). Similar results were found for the other essential and non-essential AAs, which suggests that green protein concentrates extracted from lucerne and red clover have great potential as a protein source for pigs.

In conclusion, the present study provides original data on the energy and protein values of legume silages and protein pastes in swine. Similar to others studies published on this topic, the use of constant inclusion rates of each product to be tested did not allow us to evaluate possible digestive interactions between nutrient coming from silages or proteins pastes and those from control diets. Therefore, further studies are required to validate the assumption that the inclusion rate of legume forage-based product does not have consequence in their nutritional values. In our experimental conditions, silages or protein pastes obtained from lucerne or red clover did not significantly differ for their energy values. However, based on the amount of digestibility of AA contents, red clover silage has higher protein value than lucerne silage. The very low concentration of digestible AA in lucerne silage is related to the postharvest proteolysis of native proteins during ensiling proteolysis and the reduced AA digestibility coefficients due to high DF content. Finally, in our experimental conditions, the SID values of AA were lower in RPP than LPP. As this difference is not fully explained by changes in DF, in AA linked to DF or in secondary compounds concentrations, further studies are needed to better explain why AAs from RPP are less digestible.

Ethics approval

The experiment was conducted in accordance with French legislation on animal experimentation and was approved by the French National Committee for Consideration of Ethics in Animal Experimentation (Authorisation: APAFiS #21547-2019071912373656 delivered on October 28, 2019).

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings are available upon request.

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Declaration of interest

The authors declare that they have no conflicts of interest.

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