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ORIGINAL ARTICLE

Comparison of nutritional and antinutritional traits among different species (*Lupinus albus* L., *Lupinus luteus* L., *Lupinus angustifolius* L.) and varieties of lupin seeds

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Summary

In order to promote the use of lupin in pig nutrition, in this research the nutritional characteristics (i.e. dietary fibre, alkaloid and fatty acid profile) and the *in vitro* gas production of 12 lupin varieties grown in the Mediterranean basin and belonging to three lupin species (*Lupinus albus*, *Lupinus angustifolius* and *Lupinus luteus*) were assessed. Four varieties of *L. albus* (Asfer, Lublanc, Lutteur and Multitalia) were grown in South Campania. Three varieties of *L. luteus* (Dukat, Mister and Taper), three of *L. angustifolius* (Jindalee, Sonet and Wonga) and two of *L. albus* (Rosetta and Luxor) were grown in Eastern Sicily. *Lupinus albus* varieties showed interesting nutritional and dietetic characteristics (i.e. high protein and low fibre content); the lipid fraction, rather elevated, is well represented by monounsaturated fatty acids (544 g/kg), whereas saturated fatty acids (SFAs) are less represented (167 g/kg) and the n-3/n-6 ratio (0.510) is the most favourable. *Lupinus luteus* varieties presented the most remarkable dietetic aspects, in terms of polyunsaturated fatty acid (PUFA) content (569 g/kg), n-6 PUFA series (490 g/kg), UFA/SFA (5.24) and PUFA/SFA (3.56) ratios and atherogenic (0.059) and thrombogenic (0.100) indices and very low alkaloid content (1.07 mg per 100 g). *Lupinus angustifolius* varieties showed the least interesting nutritional and dietetic characteristics: low protein and fat content, high fibre level, high SFA amount (248 g/kg) and the lowest favourable nutritional indices (IA: 0.164 and IT: 0.334). Regarding the fermentation process, in *L. albus*, the tendency to increase the rate of gas production during the early stages of fermentation suggests that the high presence of alkaloids did not affect the *in vitro* degradability, production of short-chain fatty acids and fermentation process, probably due to their concentration and/or water solubility. *Lupinus angustifolius* and *L. luteus* showed intermediate and slightly worse *in vitro* fermentation patterns respectively. From a nutritional and dietetic point of view, lupin may represent an interesting alternative to soya bean in pig feeding.

Keywords dietary fibre, alkaloids, fatty acids, nutritional indices, *in vitro* fermentation, pig

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Introduction

In human nutrition, legumes are the main source of vegetable protein (Kohajdová et al., 2011). In animal nutrition, especially in intensive livestock systems, soya bean is the most utilized protein source, mainly administered as meal solvent extract (s.e.), a by-product of the oil industry, where soya bean seeds are treated with high temperature and organic solvents. However, recently some obstacles are limiting the use of soya bean: the ban in organic livestock (EC Council Regulation 834/2007) due to the chemical treatment and its costs and availability strongly related with the price development of agricultural commodities on the world market. In Italy, there is an increasing interest in the use of Mediterranean legume grains, which are also important because they increase the sustainability of livestock systems (Calabrò et al., 2009, 2015; Gresta et al., 2010), contributing to the improvement of soil structure, fixing atmospheric nitrogen to the soil and reducing the fertilization costs. Among these, lupin may represent a very interesting alternative to soya bean. There are more than 170 lupin species (Gresta et al., in press), but the most interesting for grain

production and animal feeding are a lot fewer. In fact, cultivated species of lupin used as feed ingredient for pigs, ruminants and poultry mainly include *Lupinus albus* L., *Lupinus angustifolius* L. and *Lupinus luteus* L., all originating from the Mediterranean area. The lupin seed is a protein source used throughout the world, due to its interesting agronomic characteristics (i.e. hardiness to be grown in soils and climates other than native; Kohajdová et al., 2011) and also for its nutritional value. Lupin seeds are also rich in dietary fibre (Rochfort and Panozzo, 2007) and contain minor compounds such as lipids, polyphenols and bioactive peptides (Pastor-Cavada et al., 2009). As reported by Erbas et al. (2005), the oil, with a variable content from 6 to 13% depending on the species, has an interesting fatty acid profile with a high concentration of unsaturated fatty acids (UFAs) (Duranti et al., 2008), characterized by a high level of alpha-linolenic acid and a favourable n-3/n-6 fatty acid ratio (Chiofalo et al., 2011). Their common drawback is the low content of sulphur amino acids (methionine and cystine) and antinutritional factors (ANFs), mainly represented by quinolizidine alkaloids, sparteine and lupanine, which can reduce animal performances, and can cause respiratory arrest. Godfrey et al. (1985) examined a range of lupin alkaloid concentrations in pig diets and found that growing pigs could tolerate up to 0.2 g/kg of dietary lupin alkaloids before feed intake would be reduced. Therefore, the use of lupin is closely related to the reduction in alkaloids (Dronne, 2003), whose content can be reduced by selecting sweet genetic varieties with low alkaloid content or by adopting treatments like soaking in running water, brine or scalding. Because of their high protein and oil levels and their interesting type of fibre, they are a potentially valuable protein and energy supplement to cereal-based pig diets.

Data concerning the utilization of lupin in pig nutrition are ambiguous. Feeding diets containing 150 to 430 g/kg of *L. albus* seeds reduced live weight gains (Zettl et al., 1995) and feed intake (Van Nevel et al., 2000) of pigs. On the contrary, no growth depression was found by Gdala et al. (1996) in pigs fed with a diet containing *L. angustifolius* (410 g/kg of diet) versus a diet based on barley and soya. Flis et al. (1996) obtained positive results with the yellow lupin variety Juno. Fernández and Batterham (1995) reported that the lupin seed meal was superior in terms of growth and health promotion than soya bean meal in growing and in finishing pigs. Hanczakowska and Świątkiewicz (2014) reported that a mixture of rapeseed press cake with lupin seeds, used in the first fattening and finisher period, could replace soya bean meal in fattening pigs without lowering body weight gains and carcass and meat quality. Comparing different varieties of lupin (Lublanc, Amiga and Boltensia), Froidmont et al. (2005) suggested that Lublanc variety was the most suitable for growing and finishing pigs.

The hypothesis is that lupin species and varieties may affect nutritive values and *in vitro* fermentation characteristics and kinetics. Moreover, it is supposed that the *in vitro* parameters are correlated to the presence of some compounds (i.e. alkaloids, fatty acids and fibre fractions).

In order to promote the use of lupin in pig nutrition, the aim of the present research was to assess the nutritional characteristics (i.e. dietary fibre, alkaloid and fatty acid profile) and the *in vitro* gas production (IVGP) of 12 sweet lupin varieties, grown in the Mediterranean basin and belonging to three lupin species. The use of the IVGP technique (IVGPT) is largely used in ruminant nutrition to estimate the feed nutritive value and study their fermentation kinetics (Musco et al., 2016). Recently, IVGPT was employed in pig studies (Bauer et al., 2003; Musco et al., 2015) to characterize the gut fermentation of different carbohydrate fractions, replacing rumen liquid by *caecal* or *faecal* material.

Materials and methods

Plant material and environmental conditions

Twelve sweet lupin varieties belonging to three species (*L. albus* L., *L. angustifolius* L. and *L. luteus* L.) cultivated in two areas of South Italy were tested. Four varieties of *L. albus* (Asfer, Lublanc, Lutteur and Multitalia) were sown in the last days of November in an experimental area of Campania (Piana del Sele, Pontecagnano, SA, Italy, altitude 28 m a.s.l., annual mean temperature ranging from 15 to 23 °C and annual average rainfall 770 mm) on a silt-clay soil with sub-alkaline pH, normal salinity, average critical salt concentrations, low traces of limestone, low organic matter (OM) and N content and high P and K content. Seeds were randomly collected at the end of April.

The other seeds, three varieties of *L. luteus* (Dukat, Mister and Taper), three varieties of *L. angustifolius* (Jindalee, Sonet and Wonga) and the last two varieties of *L. albus* (Rosetta and Luxor) were sown in the last days of December in sandy soil in Eastern Sicily, Italy (Acireale, CT, 16 m a.s.l.), with sub-alkaline pH, low salinity, good OM, low value of nitrogen and high value of P and K of P_2O_5. The seeds were harvested at the end of May. The mean annual temperature of the experimental site ranges from 16.3 °C to 23.8 °C with a rainfall of 806 mm. For both sites, after collection,

all samples were stored at 4 °C until the analyses were carried out. Overall, the total number of samples was 108: 12 (varieties) × three (parcel repetitions) × three (analytical replicates). Only field replicates were used for the statistical comparison.

Chemical composition

The samples were ground with a 1-mm screen and analysed for dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF) and ash as reported by AOAC (2005) procedures (ID number: 2001.12, 978.04, 920.39, 978.10 and 930.05 for DM, CP, EE, CF and ash respectively). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were also determined. The non-structural carbohydrates (NSCs) were calculated according to the following formula: DM − (CP + EE + NDF + Ash). The indications of Lee and Prosky (1995) were utilized to determine total dietary fibre (TDF), insoluble dietary fibre and soluble dietary fibre (SDF) contents.

Alkaloids were extracted as described by Muzquiz et al. (1994) and analysed by HRGC-MS (Nossack et al., 2000), performed using a 5973 *inert mass* selective detector (Agilent Technologies, Palo Alto, CA, USA) and coupled to a 6890N GC (Agilent Technologies). The capillary column used was a 95% methyl, 5% phenylpolysiloxane, HP-5 (Agilent J&W GC Columns) of 30 m length, 0.25 mm i.d. and 0.25 μm film thickness. The column temperature was programmed to rise from 150 °C, at 5 °C/min, to 235 °C (held during 15 min). Helium was used as carrier gas. The alkaloid quantification was performed in full-scan mode by the internal standard method using caffeine as an analytical standard. In the standard solutions, the limit of quantification (L.O.Q.; S/N > 7) was of 0.2 mg/kg for sparteine and 0.4 mg/kg for all other alkaloids (Calabrò et al., 2015).

To analyse the fatty acid profile, the lupin seeds were dehulled and ground. The flour was extracted with hexane in a Soxhlet apparatus, and the solvent was then evaporated under reduced pressure according to the method of Boschin et al. (2007). The fatty acid methyl esters of the lupin seeds were prepared by direct transesterification (Christie, 1993). The FAMEs were analysed by GC-FID (Agilent Technologies) with a split/splitless injector, a flame ionization detector and fused silica capillary column Omegawax 250 (Supelco, Bellefonte, PA, USA), 30 m × 0.25 mm I.D. and 0.25 m film thickness as described by Chiofalo et al. (2012). Identification of fatty acids was made by comparing the relative retention times of FAME peaks from samples with standards from Supelco (Bellefonte, PA, USA). Chromatogram peak areas were acquired and calculated by Chemstation software (Agilent). The concentrations of individual fatty acids were expressed in g/kg of the total fatty acid methyl esters identified. Fatty acids were grouped into saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). For each sample, the chromatographic analysis was replicated three times.

Owing to the relevance for human health, based on the identified fatty acids, the Atherogenic Index (AI) and the Thrombogenic Index (TI) were calculated using the equations proposed by Ulbricht and Southgate (1991).

In vitro gas production technique

The fermentation characteristics and kinetics were studied using the IVGPT as reported by Musco et al. (2015), by incubating all the lupin samples (0.5007 ± 0.0005 g) in triplicate, at 39 °C under anaerobic condition with buffered *caecal* fluid collected at a slaughterhouse approved by EU from six adult neutered finisher pigs (Landrace × Large White) fed a commercial diet (CP: 14.8%; CF: 4.0% as fed). Three flasks with no substrate were incubated as blanks to correct for organic matter (dOM) degradability and gas and end products.

Gas production of fermenting cultures was recorded 17 times during the incubation using a manual pressure transducer (Cole and Parmer Instrument, Vernon, IL, USA). The fermentation was stopped at 48 h, and the fermentation liquor was analysed for pH with a pH meter (model 3030 Alessandrini Instrument glass electrode, Jenway, Dunmow, UK) and sampled for end product analysis. Short-chain fatty acids (SCFAs, mM/g incubated OM) were measured (Cutrignelli et al., 2009) by gas chromatography (model. 8000 top; ThermoQuest Italia SpA, Rodano, Milan, Italy); ammonia (NH_3, mM/g incubated OM) was determined according to the colorimetric method (Musco et al., 2016).

The dOM (% of incubated OM) was determined by filtering the residues throughout pre-weighed sintered glass crucibles (Scott Duran, porosity #2) under vacuum, drying to a constant weight at 103 °C and burning for 5 h at 550 °C.

Data processing

Cumulative volume of gas produced after 48 h of incubation was related to incubated OM (OMCV,

ml/g) and degraded OM (Yield, ml/g). For each flask, the gas production data were processed as reported by Musco et al. (2015) using the sigmoid model:

$$G = \frac{A}{\left(1 + \left(\frac{B}{t}\right)\right)^{C}}$$

where G is the total gas produced (ml/g of incubated OM) at time t (h), A is the asymptotic gas production (ml/g of incubated OM), B (h) is the time at which one-half of the asymptote is reached and C is the switching characteristic of the curve. Maximum fermentation rate (R_{max}, ml/h) and the time at which it occurred (T_{max}, h) were also calculated using the formulas:

$$R_{max}(\text{ml/h}) = \frac{(AC^{B})B[T_{max}^{(-B-1)}]}{[(1 + C^{B})\,(T_{max}^{-B})^{2}]}$$

$$T_{max}(\text{h}) = C\left[\frac{(B-1)}{(B+1)}\right]^{(1/B)}$$

Chemical composition data and fatty acid content, fermentation characteristics (OMCV, yield, OM digestibility and pH), model parameters (A, B, T_{max}, R_{max}) and fermentation end products (SCFAs and NH_3) were subjected to nested analysis of variance to detect the influence of lupin varieties according to the model:

$$Y_z = \mu + \text{Block}_w + \text{Species}_j + (\text{Species}_j/\text{Var}_i) + \varepsilon_z,$$

where Y is each single datum (z goes from 1 to 36), μ is the grand mean, Block is the block effect (w = 1 to 3), Species is the species effect (j = 1 to 3), Species/Var is the variety effect within species (i = 1 to 6 for *L. albus* and i = 1 to 3 for *L. angustifolius* and *L. luteus*) and ε is the error term. The analysis was performed using the R (version 2.11.0) statistical environment (R Development Core Team, 2010). Tukey's test was adopted as multiple-comparison test to determine the source of variation.

The correlation between the chemical composition and fatty acid profile vs. the *in vitro* fermentation parameters was studied (PROC CORR, SAS/STAT 2000: User's Guide Version 8.2, SAS Institute, Cary, NC).

Results

Chemical composition

The chemical composition of the studied varieties grouped for *Lupinus* species is reported in Tables 1–5. Overall, some parameters resulted quite diversified between *L. albus*, *L. luteus* and *L. angustifolius*. In particular, CP and lipid values resulted significantly ($p < 0.01$) higher in *L. albus* and lower in *L. angustifolius* (CP: 378 and 290 g/kg DM; EE: 86.7 and 39.8 g/kg DM respectively). Regarding the carbohydrates, *L. albus* showed the lowest ($p < 0.01$) values in terms of cell wall, NSCs and TDF (NDF: 206, NSC: 195 and TDF: 466 g/kg DM). Regarding dietary fibre, in all samples the insoluble fraction were more than 80% of the TDF, with the exception of variety Dukat (77.1% TDF), whereas the soluble fraction was significantly ($p < 0.01$) higher in *L. luteus*; very low value was observed in Rosetta (SDF: 8.94% TDF). Comparing varieties within species, many interesting differences emerged. For *L. albus*, Multitalia showed the highest ($p < 0.05$) CP and ADL values, whereas Asfer showed the lowest ($p < 0.05$) NDF value. Within L. *luteus* varieties, Dukat showed the highest ($p < 0.05$) EE and NDF contents; few differences resulted in CP content. Regarding *L. angustifolius* varieties, Sonet showed the highest ($p < 0.05$) EE and Wonga the highest ($p < 0.05$) NDF, ADF and TDF contents.

As reported in Table 2, six alkaloids were detected and quantified: sparteine, lupanine, angustifoline, alpha-isolupanine, 13-alpha-hydroxylupanine and 11,12-deidrelupanine. The chromatographic analysis allowed the quantification of six alkaloids. Moreover, among these six alkaloids, the chromatographic analysis did not allow identification of their presence, especially in the varieties of *L. luteus* and *L. angustifolius*, because their content was below the instrumental limit of quantification (0.04 mg per 100 g; Table 2). Very low concentrations of quinolizidine alkaloids were observed in all the varieties, except for Multitalia, the historical Italian variety, which showed the significantly highest ($p < 0.05$) value (153 mg per 100 g) among the varieties of *L. albus*. Within this species, lupanine (34.6 mg per 100 g) was the most represented alkaloid followed by 13-alpha-hydroxylupanine (2.54 mg per 100 g), angustifoline (1.286 mg per 100 g), 11,12-deidrelupanine (1.122 mg per 100 g), alpha-isolupanine (0.465 mg per 100 g) and sparteine 0.107 mg per 100 g).

Among the varieties of the *L. luteus*, no significant differences ($p > 0.05$) in the total alkaloids were observed for Dukat (0.965 mg per 100 g), Mister (0.873 mg per 100 g) and Taper (1.36 mg per 100 g). Sparteine (1.012 mg per 100 g) was, on average, the most represented alkaloid, followed by 13-alpha-hydroxylupanine (0.11 mg per 100 g) and lupanine (0.053 mg per 100 g).

Table 1 Chemical composition in the seeds of three lupin species

Variety	DM	Ash	CP	EE	NDF	ADF	ADL	NSC	TDF	IDF	SDF
	g/kg DM									% TDF	
Asfer	912	57.4^{b}	383^{c}	92.1^{c}	189^{c}	180^{a}	27.4^{d}	191^{c}	481^{c}	83.77^{bc}	14.29^{ab}
Lublanc	914	45.7^{c}	409^{b}	106^{a}	217^{a}	95.9^{c}	32.9^{c}	137^{e}	415^{e}	87.35^{b}	12.65^{b}
Lutteur	920	46.0^{c}	396^{bc}	91.0^{c}	213^{a}	162^{b}	30.4^{cd}	174^{d}	460^{d}	84.29^{c}	15.71^{a}
Luxor	910	55.0^{b}	319^{d}	75.5^{d}	206^{b}	178^{a}	41.4^{b}	259^{b}	505^{b}	83.41^{c}	16.59^{a}
Multitalia	908	43.5^{c}	454^{a}	99.0^{b}	213^{a}	99.0^{c}	49.0^{a}	99.0^{f}	417^{e}	83.77^{c}	16.23^{a}
Rosetta	913	67.5^{a}	309^{d}	70.4^{e}	203^{b}	184^{a}	42.1^{b}	312^{a}	521^{a}	91.06^{a}	8.94^{c}
Lupinus albus	913	52.5^{B}	378^{A}	86.7^{A}	206^{B}	150^{C}	36.7^{A}	195^{C}	466^{C}	85.93^{A}	14.07^{B}
Dukat	911	64.0^{a}	343^{ab}	59.2^{a}	248^{a}	213^{a}	19.4^{ab}	197^{c}	508^{a}	77.09^{b}	22.91^{a}
Mister	909	40.0^{b}	362^{a}	51.8^{c}	232^{b}	201^{b}	14.9^{b}	224^{b}	496^{b}	80.27^{a}	19.73^{b}
Taper	905	66.0^{a}	322^{b}	55.7^{b}	217^{c}	195^{c}	23.8^{a}	244^{a}	513^{a}	80.98^{a}	19.02^{b}
Lupinus luteus	908	56.7^{A}	342^{B}	55.3^{B}	232^{B}	204^{B}	17.5^{B}	222^{B}	505^{B}	79.5^{B}	20.60^{A}
Jindalee	905	30.7^{b}	303^{a}	43.2^{b}	274^{b}	240^{b}	34.7^{a}	313^{b}	574^{b}	89.08^{a}	10.91^{b}
Sonet	900	46.8^{a}	277^{b}	46.3^{a}	257^{c}	236^{b}	28.7^{b}	332^{a}	567^{b}	83.47^{b}	16.53^{a}
Wonga	903	46.9^{a}	289^{ab}	32.8^{c}	312^{a}	276^{a}	37.0^{a}	281^{c}	588^{a}	86.19^{ab}	13.81^{ab}
Lupinus angustifolius	903	41.5^{C}	290^{C}	39.8^{C}	281^{A}	250^{A}	31.8^{A}	309^{A}	576^{A}	86.3^{A}	13.80^{B}

DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; NSC, non-structural carbohydrates; TDF, total dietary fibre; IDF, insoluble dietary fibre; SDF, soluble dietary fibre.
Along the column, different capital letters indicate significant differences among species, and different lowercase letters indicate differences among varieties within each species.

Table 2 Composition of quinolizidine alkaloids (mg/100 g) in the seeds of three lupin species

Variety	Sparteine	Lupanine	Angustifoline	Alpha-isolupanine	13-Alpha-Hydroxylupanine	11,12-Deidrelupanine	Total
Asfer	ND	3.33 ± 0.04	ND	ND	ND	0.45 ± 0.02	3.78^{c}
Lublanc	0.026 ± 0.00	56.1 ± 0.25	0.754 ± 0.02	0.467 ± 0.01	2.58 ± 0.03	ND	63.4^{b}
Lutteur	0.096 ± 0.01	8.92 ± 0.51	0.623 ± 0.09	0.228 ± 0.03	1.90 ± 0.03	ND	13.2^{c}
Luxor	ND	3.30 ± 0.20	0.630 ± 0.13	0.25 ± 0.02	1.34 ± 0.76	2.00 ± 0.10	7.52^{c}
Multitalia	0.200 ± 0.01	134 ± 3.60	3.93 ± 0.11	1.200 ± 0.15	6.50 ± 0.24	ND	153^{a}
Rosetta	ND	1.80 ± 0.0	0.490 ± 0.02	0.18 ± 0.01	0.380 ± 0.29	0.920 ± 0.04	3.77^{c}
Lupinus albus	0.107	34.6	1.286	0.465	2.54	1.122	38.8
Dukat	0.965 ± 0.11	ND	ND	ND	ND	ND	0.965
Mister	0.71 ± 0.03	0.05 ± 0.01	ND	ND	0.11 ± 0.01	ND	0.873
Taper	1.36 ± 0.16	ND	ND	ND	ND	ND	1.36
Lupinus luteus	1.012	0.053	–	–	0.11	–	1.07
Jindalee	ND	1.79 ± 0.01	1.12 ± 0.21	ND	2.60 ± 0.20	ND	5.51^{a}
Sonet	0.059 ± 0.21	1.19 ± 0.31	ND	ND	0.870 ± 0.2	ND	2.12^{b}
Wonga	ND	0.74 ± 0.21	0.23 ± 0.105	ND	0.560 ± 0.02	ND	1.53^{b}
Lupinus angustifolius	0.059	1.24	0.449	–	1.34	–	3.05

ND = value below the L.O.Q.
Along the column, different lowercase letters indicate differences among varieties within each species.

Among *L. angustifolius*, Jindalee showed the highest ($p < 0.05$) content of total alkaloids (5.51 mg per 100 g). On average, 13-alpha-hydroxylupanine (1.34 mg per 100 g) showed the highest content followed by lupanine (1.24 mg per 100 g), angustifoline (0.449 mg per 100 g) and sparteine (0.059 mg per 100 g).

Tables 3–5 report the SFAs, unsaturated fatty acids and the main class of acids, respectively, in the three species of *Lupins*. Among the 10 SFAs (Table 3), palmitic acid (C16:0) was found, on average, at the highest levels ($p < 0.01$) in *L. angustifolius* (113.7 g/kg) followed by *L. albus* (79.8 g/kg) and by *L. luteus* (42.7 g/kg); also, stearic acid (C18:0) showed, on average, the highest levels ($p < 0.01$) in the *L. angustifolius* (64.5 g/kg), whereas its content was similar ($p > 0.05$) in *L. albus* (21.6 g/kg) and in *L. luteus* (17.9 g/kg); the mean values of behenic acid (C22:0)

Table 3 Saturated fatty acid content (g/kg)* in the seeds of three lupin species

Variety	C13	C14	C15	C16	C17	C18	C20	C22	C23	C24
Asfer	–	1.05^{c}	0.70^{ab}	86.5^{a}	0. 60^{b}	19.8^{bcd}	10.6^{c}	35.9^{ab}	1.00^{b}	9.10^{b}
Lublanc	–	1.29^{b}	0.64^{c}	79.6^{b}	0.51^{c}	21.3^{bc}	12.4^{a}	43.4^{a}	–	10.9^{b}
Lutteur	–	0.84^{d}	0.67^{bc}	83.7^{a}	0.58^{bc}	29.4^{a}	11.8^{b}	33.0^{b}	–	10.1^{b}
Luxor	0.24	1.53^{a}	0.68^{bc}	77.6^{b}	0.58^{bc}	16.9^{d}	10.6^{c}	37.5^{ab}	4.80^{a}	15.5^{a}
Multitalia	–	0.98 ^{cd}	0.59^{d}	72.7^{c}	0.50^{c}	23.2^{b}	11.6^{b}	44.1^{a}	–	10.8^{b}
Rosetta	0.23	1.52^{a}	0.72^{a}	78.7^{b}	0.71^{a}	18.6^{cd}	11.3^{b}	41.9^{a}	3.05^{a}	15.4^{a}
Lupinus albus	0.23^{B}	1.20^{C}	0.67^{B}	79.8^{B}	0.58^{B}	21.6^{B}	11.4^{B}	39.3^{B}	2.95^{C}	12.0^{A}
Dukat	0.24	1.69^{ab}	0.63^{a}	44.1^{a}	0.65	19.7	25.2^{a}	65.6	3.66	7.34
Mister	0.26	1.86^{a}	0.58^{b}	40.7^{b}	0.57	17.9	22.9^{b}	64.9	4.14	8.15
Taper	0.22	1.51^{b}	0.59^{b}	43.4^{a}	0.61	16.2	18.7^{c}	57.4	4.48	7.96
Lupinus luteus	0.24^{B}	1.69^{B}	0.60^{C}	42.7^{C}	0.61^{B}	17.9^{B}	22.3^{A}	62.7^{A}	4.09^{B}	7.82^{B}
Jindalee	0.42^{a}	2.55^{a}	0.94^{b}	121^{a}	0.87	77.4^{a}	13.1^{a}	30.2^{b}	6.05^{b}	13.6^{b}
Sonet	0.29^{b}	2.21^{b}	0.52^{c}	111^{b}	0.68	51.0^{c}	7.9^{c}	29.0^{b}	2.94^{c}	4.33^{c}
Wonga	0.44^{a}	2.63^{a}	1.30^{a}	108^{c}	1.03	65.1^{b}	11.3^{b}	43.4^{a}	10.5^{a}	22.5^{a}
Lupinus angustifolius	0.39^{A}	2.46^{A}	0.92^{A}	113.7^{A}	0.86^{A}	64.5^{A}	10.8^{B}	34.2^{B}	6.51^{A}	13.5^{A}

Along the column, different capital letters indicate significant differences among species, and different lowercase letters indicate differences among varieties within each species.

*****The concentrations of individual fatty acids were expressed per total fatty acid methyl esters identified.

Table 4 Unsaturated fatty acid content (g/kg)* in the seeds of three lupin species

Variety	C16:1	C17:1	C18:1n9	C18:1n7	C18:2n6	C18:3n3	C20:1n9	C20:1n7	C20:2n6	C22:1n9
Asfer	4.70^{ab}	0.80^{a}	471^{b}	31.6^{a}	188^{c}	88.8^{c}	35.0^{b}	–	–	13.9^{b}
Lublanc	4.39^{bc}	0.62^{c}	467^{bc}	24.7^{bc}	176^{d}	94.6^{b}	40.1^{a}	1.05^{b}	3.27^{a}	18.7^{ab}
Lutteur	4.71^{ab}	0.71^{b}	491^{a}	26.7^{b}	188^{c}	85.8^{d}	24.9^{c}	0.84^{b}	1.90^{c}	6.11^{c}
Luxor	4.10^{c}	0.62^{c}	460^{c}	18.4^{d}	196^{b}	101^{a}	34.0^{b}	1.51^{b}	2.79^{b}	16.5^{ab}
Multitalia	4.75^{a}	0.66^{bc}	466^{bc}	22.3^{c}	178^{d}	96.0^{b}	41.5^{a}	1.15^{b}	3.58^{a}	21.5^{a}
Rosetta	3.00^{d}	0.63^{c}	439^{d}	12.1^{e}	226^{a}	90.1^{c}	34.1^{b}	3.19^{a}	3.63^{a}	17.2^{ab}
Lupinus albus	4.27^{A}	0.67^{A}	465^{A}	22.6^{A}	192^{C}	92.7^{A}	34.9^{A}	1.55^{B}	3.03^{A}	15.7^{A}
Dukat	0.69	0.51	223^{b}	3.96	480^{b}	79.7^{b}	20.6^{a}	2.92	2.92	17.2^{ab}
Mister	0.77	0.49	242^{a}	4.65	473^{c}	67.4^{c}	21.7^{a}	3.55	3.06	22.2^{a}
Taper	0.97	0.54	204^{c}	3.93	505^{a}	94.6^{a}	18.1^{b}	3.36	3.10	14.6^{b}
Lupinus luteus	0.81^{B}	0.51^{B}	223^{C}	4.18^{B}	486^{A}	80.6^{B}	20.1^{B}	3.28^{A}	3.03^{A}	18.0^{A}
Jindalee	1.00^{a}	0.41	369^{a}	5.35	287^{b}	59.0^{c}	4.40	2.15	0.55	4.70
Sonet	0.47^{b}	0.32	282^{c}	4.22	432^{a}	62.9^{b}	3.60	1.02	0.45	4.00
Wonga	1.17^{a}	0.41	348^{b}	5.53	286^{b}	76.6^{a}	4.50	2.16	0.78	8.24
Lupinus angustifolius	0.88^{B}	0.38^{C}	333^{B}	5.04^{B}	335^{B}	66.2^{C}	4.19^{C}	1.77^{B}	0.59^{B}	6.35^{B}

Along the column, different capital letters indicate significant differences among species, and different lowercase letters indicate differences among varieties within each species.

*The concentrations of individual fatty acids were expressed per total fatty acid methyl esters identified.

were higher in L. *luteus* (62.7 g/kg) than in *L. albus* (39.3 g/kg) and *L. angustifolius* (34.2 g/kg).

Seven MUFAs were identified and quantified (Table 4), and, among the three species, the most represented were oleic acid (C18:1n9) and gadoleic acid (C20:1n9), showing significantly ($p < 0.01$) higher levels in *L. albus* (465 and 34.9 g/kg, respectively) than those of the *L. luteus* (223 and 20.1 g/kg, respectively) and *L. angustifolius* (333 and 4.19 g/kg respectively). The average relative content of erucic acid was higher ($p < 0.01$) in *L. luteus* (18.0 g/kg) and *L. albus* (15.7 g/kg) than that found in *L. angustifolius* (6.35 g/kg). Within *L. albus*, Multitalia, Lublanc and Rosetta showed significantly ($p < 0.05$) higher levels of erucic acid; for the *L. luteus*, Mister and Taper showed significantly ($p < 0.05$) higher levels of erucic acid, whereas for the *L. angustifolius*, no significant differences ($p < 0.05$) of erucic acid were observed between varieties. Among the PUFAs detected, linoleic acid (C18:2n6) was the most represented in all the species, with a significantly ($p < 0.01$) higher mean content in *L. luteus* (486 g/kg), than that found in *L. angustifolius*

Table 5 Fatty acid classes (g/kg), ratios and quality indices in the seeds of three lupin species

Variety	SFA	MUFA	PUFA	UFA	n-3 PUFA	n-6 PUFA	UFA/SFA	PUFA/SFA	n-3/n-6	AI	IT
Asfer	165	557^{a}	277^{c}	834	124^{a}	188^{cd}	5.05	1.68^{bc}	0.658^{a}	0.105	0.143^{c}
Lublanc	170	557^{a}	273^{c}	830	94.6^{c}	179^{e}	4.88	1.61^{c}	0.529^{b}	0.102	0.156^{b}
Lutteur	168	555^{a}	275^{c}	830	85.8^{e}	190^{c}	4.71	1.56^{c}	0.452^{d}	0.105	0.180^{a}
Luxor	165	537^{b}	299^{b}	836	101^{b}	198^{b}	5.08	1.82^{ab}	0.507^{c}	0.099	0.140^{c}
Multitalia	165	558^{a}	278^{c}	835	96.0^{c}	182^{de}	5.08	1.69^{bc}	0.528^{b}	0.092	0.146^{c}
Rosetta	172	509^{c}	320^{a}	829	90.1^{d}	230^{a}	4.82	1.86^{a}	0.392^{e}	0.102	0.154^{b}
Lupinus albus	167^{B}	544^{A}	287^{C}	831^{A}	98.5^{A}	194^{C}	4.96^{B}	1.71^{B}	0.510^{A}	0.100^{B}	0.154^{B}
Dukat	169^{a}	269^{b}	563^{b}	831^{b}	79.7^{b}	483^{b}	4.93^{b}	3.33^{b}	0.165^{b}	0.061	0.106
Mister	162^{a}	295^{a}	543^{c}	838^{b}	67.4^{c}	476^{b}	5.17^{b}	3.35^{b}	0.142^{c}	0.057	0.103
Taper	151^{b}	246^{c}	603^{a}	849^{a}	94.6^{a}	509^{a}	5.62^{a}	3.99^{a}	0.186^{a}	0.058	0.092
Lupinus luteus	160^{C}	269^{C}	569^{A}	839^{A}	80.6^{B}	488^{A}	5.24^{A}	3.56^{A}	0.160^{C}	0.059^{A}	0.100^{C}
Jindalee	267^{a}	387^{a}	347^{c}	736^{b}	59.0^{c}	289^{b}	2.76^{b}	1.30^{b}	0.205^{b}	0.179^{a}	0.390^{a}
Sonet	210^{b}	295^{c}	495^{a}	790^{a}	62.9^{b}	432^{a}	3.76^{a}	2.35^{a}	0.146^{c}	0.152^{b}	0.297^{b}
Wonga	266^{a}	370^{b}	364^{b}	734^{b}	76.6^{a}	287^{b}	2.76^{b}	1.36^{b}	0.267^{a}	0.162^{b}	0.314^{b}
Lupinus angustifolius	247^{A}	350^{B}	401^{B}	725^{B}	66.2^{C}	335^{B}	3.09^{C}	1.67^{C}	0.210^{B}	0.160^{B}	0.334^{A}

SFA (saturated fatty acid) = C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0; MUFA (monounsaturated fatty acid) = C16:1 + C17:1 + C18:1n9 + C18:1n7 + C20:1n9 + C20:1n7 + C22:1n9; PUFA (polyunsaturated fatty acid) = C18:2n6 + C18:3n3 + C20:2n6; n-3 PUFA, n-3 polyunsaturated fatty acid; n-6 PUFA, n-6 polyunsaturated fatty acid; UFA/SFA, unsaturated/saturated fatty acid ratio; PUFA/SFA, polyunsaturated/saturated fatty acid ratio; n-3/n-6, n-3/n-6 polyunsaturated fatty acid ratio; AI, Atherogenic Index; TI, Thrombogenic Index.
Along the column, different capital letters indicate significant differences among species, and different lowercase letters indicate differences among varieties within each species.

and in *L. albus* (335 and 192 g/kg, respectively) followed by the mean content of alpha-linolenic acid (C18:3n3) found in significantly ($p < 0.01$) greater quantity in the *L. albus* (92.7 g/kg) than that found in *L. luteus* and in *L. angustifolius* (80.6 and 66.2 g/kg respectively).

As regards the essential fatty acids, *L. albus* showed the significantly ($p < 0.05$) higher content of linoleic acid in Rosetta (226 g/kg) and alpha-linolenic acid in Luxor (101 g/kg); *L. luteus* showed the significantly ($p < 0.05$) higher content of linoleic acid in Taper (505 g/kg) and alpha-linolenic acid in Dukat (20.6 g/kg); *L. angustifolius* showed the significantly ($p < 0.05$) higher content of linoleic acid in Sonet (432 g/kg) and alpha-linolenic acid in Wonga (76.6 g/kg).

Table 5 covers the fatty acid classes, the fatty acid ratios and the quality indices, AI and TI. From a nutritional point of view, *L. luteus* (160 g/kg, on average) showed significantly ($p < 0.01$) lower values of SFAs than those of *L. angustifolius* (247 g/kg, on average) and *L. albus* (167 g/kg, on average). As regards MUFAs, *L. albus* showed the significantly ($p < 0.01$) highest values (544 g/kg, on average), followed by the varieties of *L. angustifolius* and *L. luteus* (350 and 269 g/kg respectively). Concerning the total PUFAs, *L. luteus* showed the significantly ($p < 0.01$) highest values (569 g/kg, on average), followed by the varieties of *L. angustifolius* and *L. albus* (401 and 287 g/kg respectively). Among PUFAs, those of the n-6 series were significantly ($p < 0.01$) higher in *L. luteus* (488 g/kg) and those of the n-3 series in *L. albus* (98.5 g/kg). Consequently, the UFA/SFA ratio showed significantly ($p < 0.01$) higher values in *L. luteus* (5.24) followed by *L. albus* (4.96) and *L. angustifolius* (3.09). The PUFA/SFA ratio was significantly ($p < 0.01$) higher in *L. luteus* (3.56) than that found in *L. albus* (1.70) and *L. angustifolius* (1.67). Finally, the n-3/n-6 ratio, a very interesting ratio from a nutritional point of view, was found at the highest value in *L. albus* (0.51), followed by *L. angustifolius* (0.21) and *L. luteus* (0.16).

Consequently, AI and TI, strictly related to the fatty acid profile, showed significantly ($p < 0.01$) lower and therefore better values in *L. luteus* (0.059 and 0.100 respectively).

In vitro fermentation characteristics

Regarding the fermentation characteristics (Table 6), all the values resulted significantly ($p < 0.05$) different between varieties only in *L. albus*, except T_{max} parameter; in particular, Luxor showed the highest ($p < 0.05$) OMCV, *A* and *B* values. On comparing species, *L. albus* showed the highest organic matter degradability value (dOM: 83.6%) compared to the other two lupin species. The gas production resulted in every cases (OMCV, yield, A) higher in *L. angustifolius* (151.7, 192.4, 170.9 ml/g, respectively), even if the differences were not significant. All the samples

Table 6 *In vitro* fermentation characteristics in the seeds of three lupin species

Variety	dOM %	OMCV ml/g	Yield ml/g	A ml/g	B h	T_{max} h	R_{max} ml/h
Asfer	88.2^{a}	147.0^{b}	166.7^{ab}	155.0^{b}	13.01^{b}	7.76	7.87^{b}
Lublanc	88.9^{a}	140.4^{b}	157.9^{b}	143.7^{b}	11.12^{b}	6.75	8.62^{a}
Lutteur	85.6^{a}	141.6^{b}	165.6^{ab}	152.0^{b}	12.82^{b}	6.82	7.69^{b}
Luxor	75.6^{b}	166.0^{a}	206.4^{a}	185.3^{a}	15.65^{a}	8.34	7.61^{b}
Multitalia	87.7^{a}	143.4^{b}	156.1^{b}	148.4^{b}	12.41^{b}	7.63	8.00^{ab}
Rosetta	74.4^{b}	147.8^{b}	200.4^{ab}	158.6^{b}	13.10^{b}	6.30	7.52^{b}
Lupinus albus	83.6^{A}	147.7	175.5	157.1	13.02^{B}	7.27^{A}	7.88^{A}
Dukat	79.7	140.2	176.2	159.2	14.05	4.69	6.92
Mister	72.5	136.9	194.2	152.2	14.04	5.20	6.70
Taper	73.0	138.3	189.8	151.5	13.11	4.90	7.06
Lupinus luteus	75.1^{B}	138.5	186.7	154.3	13.73^{AB}	4.93^{B}	6.89^{B}
Jindalee	75.4	150.3	199.4	171.9	14.00	5.26	7.48
Sonet	81.1	151.2	186.5	165.6	14.14	6.20	7.19
Wonga	80.4	153.7	191.2	175.1	15.35	5.31	6.85
Lupinus angustifolius	79.0^{AB}	151.7	192.4	170.9	14.50^{A}	5.59^{B}	7.17^{B}

dOM = organic matter digestibility (% of incubated OM); OMCV = cumulative volume of gas related to incubated OM (ml/g); Yield = cumulative volume of gas related to degraded OM; A = potential gas production (ml/g); B = time at which A/2 was formed (h); T_{max} = time at which maximum rate was reached (h); R_{max} = maximum fermentation rate (ml/h).
Along the column, different capital letters indicate significant differences among species, and different lowercase letters indicate differences among varieties within each species.

reached the half potential gas production (B) after about 13 h of incubation.

Figure 1 shows the fermentation rate representation for the three species. The six *L. albus* varieties showed the more elevated fermentation rate values (R_{max}: 7.88 ml/h; $p < 0.01$), reached in slower time (T_{max}: 7.27 h; $p < 0.01$) compared to the other species. In particular, Lublanc stands out for the significantly highest fermentation rate (R_{max}: 8.62 ml/h; $p < 0.05$) in very short time (T_{max}: 6.75 h); then, the fermentation rate radically decreased; also, the other varieties showed similar profiles. All the *L. luteus* varieties showed lower values of fermentation rate (R_{max}: 6.89 ml/h), more evident in Mister reached in shorter time (T_{max}: 4.93 h). The varieties of *L. angustifolius* appeared close in terms of fermentation profile and comparing their values among species show intermediate values.

Regarding the fermentation end products (Table 7), the tested parameters showed statistically significant differences ($p < 0.05$) in *L. albus*, whereas only few parameters (valeric acid and SCFA) in *L. angustifolius;* in *L. luteus*, all the parameters were statistically different among varieties except pH and butyric acid. The total SCFAs seemed very similar for the three species (mean values: 149, 142 and 145 mM/g iOM for *L. albus*, *L. luteus* and *L. angustifolius* respectively). The acetic acid production, the most representative volatile fatty acid, resulted equal to 86.0, 86.1 and 88.1 mM/g iOM in *L. albus*, *L. luteus* and *L. angustifolius* respectively. The propionic acid resulted lower in the *L. luteus* (27.7 mM/g iOM) compared to the others. *Lupinus albus* recorded a higher ($p < 0.01$) production of butyric acid compared to the other two species (16.0, 12.9 and 13.8 mM/g iOM for *L. albus*, *L. luteus* and *L. angustifolius* respectively). The less representative fatty acids (isobutyric, isovaleric and valeric) were higher in *L. albus*, even if only for the isovaleric acid, the difference was significant ($p < 0.01$). On comparing varieties within species, no univocal results emerged. However, Multitalia exhibited the highest values for most of the fatty acids, whereas Rosetta the lowest.

The pH values range between 6.65 and 6.77 in Luxor and Multitalia varieties respectively. The NH_3 production seems not to be affected by the lupin species (mean values: 10.3, 10.6 and 9.7 mM/g) and ranges between 8.15 and 12.9 mM/g iOM for Luxor and Lublanc varieties respectively.

Discussion

Chemical composition

The chemical composition of all the tested lupin species is within the range reported in the literature (Abreu and Bruno-Soares, 1998; Calabrò et al., 2009) and in recent research (Calabrò et al., 2015). Fernández and Batterham (1995) reported that the CP of lupin ranged from 250 to 400 g/kg, with the exception of *L. angustifolius* (CP < 250 g/kg). These data are in

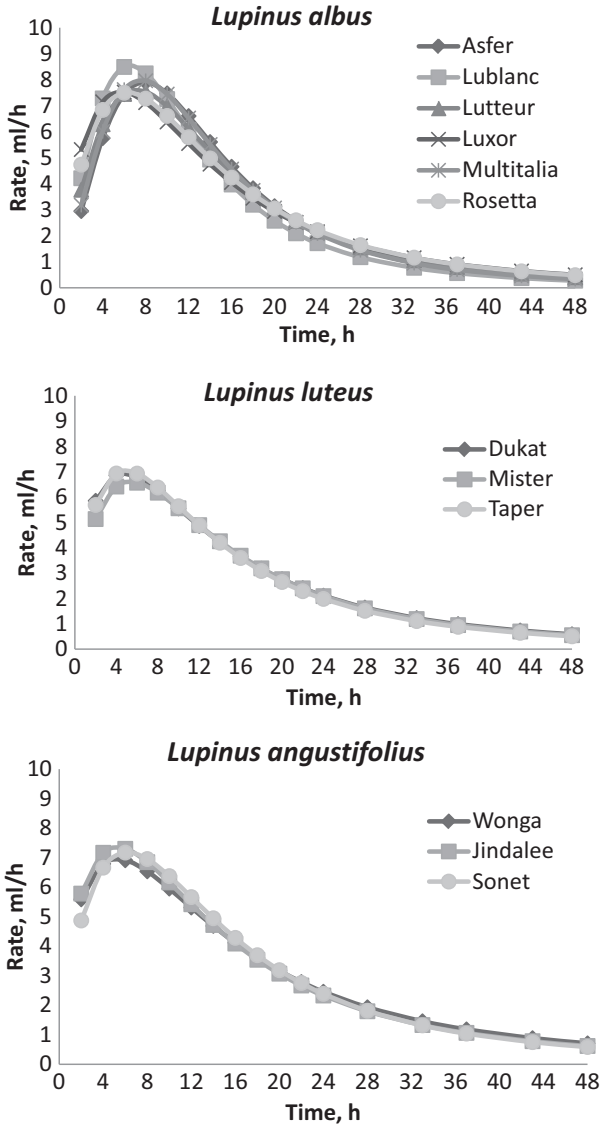

Fig. 1 *In vitro* fermentation rate over time in the seeds of three lupin species.

discordance with our data (mean values: 290 g/kg). Other authors founded a higher level in *L. luteus* compared to *L. albus:* 465 vs. 360 g/kg (Sujak et al., 2006) and 388 vs. 319 g/kg (Gdala et al., 1996) respectively.

The lupin seeds, due to the interesting lipid fraction, can represent an energy quote in pig nutrition. In the tested samples, their values are in agreement with the literature (Gdala et al., 1996; Abreu and Bruno-Soares, 1998): higher content in *L. albus* and similar in *L. luteus* and *L. angustifolius*. Compared to our data, higher lipid content is reported by Kohajdová et al. (2011) for different varieties.

As known, lupin seeds show more structural carbohydrates compared to most grain legumes, as evidenced also by the dietary fibre content. In pigs, the energy provided by the volatile fatty acids produced during the fermentation of dietary fibre in the large intestine can be substantial (Musco et al., 2015). Some fibre fractions are also exploited as prebiotics to favour the development of a beneficial microflora (Bindelle et al., 2007). Other authors (Abreu and Bruno-Soares, 1998; Aumiller et al., 2015) also found an elevated cell wall content in *L. angustifolius*. The TDF content for all the varieties was higher compared to data reported by Guillon and Champ (2002) and Kohajdová et al. (2011) and similar to Písaříková and Zralý (2010); regarding the distribution between soluble and insoluble fraction, our data are in agreement with all the above-mentioned authors.

The addition of moderately fermentable fibre sources in pig diet can reduce the production of damaging microbial metabolites in the large intestine and the incidence of intestinal complaints. Furthermore, in these conditions, the microbiota present in the large intestine keeps more nitrogen for its own growth leading to a decrease in urinary nitrogen excretion (Jha and Berrocoso, 2016).

Observing the singular variety, it is possible to observe that Multitalia showed the most valuable chemical composition in terms of CP and EE content (highest values) and structural carbohydrates, except for the lignin content (the highest values); also, the SDF is fairly represented. Conversely, Sonet variety showed the lowest values of CP and the highest level of NSCs.

Alkaloid composition

The analysis of the results showed that the studied samples had much lower alkaloid content than those reported by Reinhard et al. (2006) for *L. luteus* (500–895 μg/g), *L. angustifolius* (44–2120 μg/g) and *L. albus* (143–226 μg/g), except, for *L. albus*, for Lublanc and Multitalia sweet varieties. The Lupanine was the most represented alkaloid among the varieties in *L. albus*; these results are in agreement with the observations of Guemes-Vera et al. (2012) in the alkaloid profile of *L. albus* and with those of Boschin et al. (2008) in nine alkaloid-poor varieties of lupin seeds grown in two Italian sites.

The analysis of the results showed that *L. luteus* and *L. angustifolius* had much lower alkaloid content than the limit of toxicity (20 mg per 100 g) indicated for human and animal consumption by the health authorities of the UK, France and Australia (Boschin et al., 2008). Nevertheless, among the *L. albus*, Lublanc and Multitalia had a total content of alkaloids

Table 7 Fermentation end products in the seeds of three lupin species

Variety	pH	Acetic mM/g iOM	Propionic	Isobutyric	Butyric	Isovaleric	Valeric	SCFAs	NH_3
Asfer	6.75^{a}	87.0^{ab}	31.9^{ab}	4.00^{a}	16.8^{ab}	6.82^{b}	6.83^{a}	154^{ab}	9.71^{c}
Lublanc	6.74^{a}	84.7^{bc}	30.6^{b}	3.82^{b}	14.1^{c}	6.69^{b}	6.84^{a}	147^{c}	12.9^{a}
Lutteur	6.73^{a}	85.9^{abc}	30.1^{b}	3.79^{b}	16.9^{ab}	7.10^{ab}	5.26^{b}	149^{bc}	11.7^{b}
Luxor	6.65^{b}	89.0^{a}	30.9^{b}	3.58^{c}	16.6^{ab}	5.42^{c}	5.90^{ab}	151^{abc}	8.15^{d}
Multitalia	6.77^{a}	87.2^{ab}	33.9^{a}	4.09^{a}	17.3^{a}	7.66^{a}	6.45^{a}	157^{a}	9.77^{c}
Rosetta	6.72^{a}	83.0^{c}	26.9^{c}	3.31^{d}	14.6^{bc}	6.02^{c}	5.12^{b}	139^{d}	10.1^{c}
Lupinus albus	6.73	86.0^{B}	30.7^{A}	3.78	16.0^{A}	6.69^{A}	6.08	149	10.3
Dukat	6.72	90.7^{a}	27.2^{b}	3.37^{a}	12.6	6.37	6.35^{a}	147	11.6^{a}
Mister	6.74	84.8^{b}	30.2^{a}	3.42^{a}	14.4	5.46	6.27^{a}	145	9.96^{b}
Taper	6.70	82.2^{b}	26.6^{b}	3.14^{b}	12.3	5.32	5.39^{b}	135	10.1^{b}
Lupinus luteus	6.72	86.1^{B}	27.7^{B}	3.30	12.9^{B}	5.75^{B}	5.97	142	10.6
Jindalee	6.67	88.6	29.0	3.20	13.1	4.87	5.70	145	9.46
Sonet	6.71	87.6	30.4	3.20	14.6	4.97	5.24	146	9.50
Wonga	6.74	88.1	28.6	3.18	13.6	5.08	5.19	144	10.2
Lupinus angustifolius	6.71	88.1^{A}	29.3^{A}	3.19	13.8^{B}	4.97^{B}	5.38	145	9.70

SCFAs, short-chain fatty acids; NH_3, ammonia.

Along the column, different capital letters indicate significant differences among species, and different lowercase letters indicate differences among varieties within each species.

higher than the limit of toxicity. The reported lupanine content was too low to play any important biological activity role within human and animal metabolisms (Keeler, 1989), except for variety Lublanc and variety Multitalia. Even though a pair comparison among tested species was not possible as they were not cultivated in the same environment, on the whole, the quinolizidine alkaloids of the sweet varieties belonging to *L. albus* (Asfer, Lutteur, Luxor, Rosetta), *L. angustifolius* (Jindalee, Sonet, Wonga) and *L. luteus* (Dukat, Mister, Taper) showed considerably lower values. A large part of this difference may be ascribed to the remarkable breeding improvement of the new varieties.

In addition to saponins, tannins and alpha-galactosides, alkaloids are one of the main ANFs in lupins. As well known, pigs appear more sensitive to alkaloids compared to poultry and ruminants. The literature reports that among the most cultivated lupin species, *L. albus*, *L. angustifolius* and *L. luteus*, *L. albus* is not recommended for pig feeding as inclusion of this species delays gastric emptying and hence decreases feed intake (Kim, 2013). *Lupinus angustifolius* and *L. luteus* are the most suitable lupin species for feeding pigs. The problem is likely to be far more complex involving interactions between a combination of lupin alkaloids and other antinutritive components such as alpha-galactosides of the raffinose series, mainly stachyose, predominant among oligosaccharides of lupins, which could cause metabolic interference and metabolic troubles (Froidmont et al., 2005). In fact, inclusion of lupin in diets for growing pigs has been limited to 25% as it was thought that lupin increases the level of dietary ANFs, which compromises nutrient utilization efficiency and hence growth of pigs. Among the ANFs, alkaloid content was a concern in the past and should be monitored to limit dietary alkaloid content to 0.2 g/kg, which is considered the maximum level that maintains pig performance (Kim, 2013). Because diet formulations rarely have more than 350 g/kg of lupin seed, alkaloids only become a problem when they exceed 0.6 g/kg of seed. As alkaloid content of recent varieties of sweet lupin ranges from 0.1 to 0.4 g/kg, with a mean content of 0.2 g/kg, poor pig performance due to alkaloids in sweet lupin varieties is unlikely (Kim, 2013).

Pearson and Carr (1977) reported a poor growth and food consumption of pigs fed with a diet containing *L. albus* (variety Neuland). However, food intake and growth performance were restored reducing the alkaloid content of the seed from 0.9 g/kg to 0.2 g/kg. Also, Godfrey et al. (1985) examined a range of lupin alkaloid concentrations in pig diets and found that growing pig could tolerate up to 0.2 g/kg of dietary lupin alkaloids before feed intake was reduced. Nevertheless, the alkaloid content in the present sweet varieties of lupin was low and there have been very few reports of toxicity or feed intake depression in pigs given diets containing up to 30–40% *L. angustifolius* or *L. albus* (King, 1990) or *L. luteus*.

Fatty acid profile

Among the SFAs, mean value of the palmitic acid content of *L. albus* was similar to Uzun et al.'s (2007) observations in *L. albus* seeds (76 g/kg), while stearic acid content was similar to those reported in seeds of other legumes, which can be used in animal and human nutrition, such as *Hedysarum*, *Lathyrus*, *Gonocytisus*, *Trigonella*, *Onobrychis*, *Lens*, *Pisum*, *Astragalus* and *Vicia* (Bağci, 2006). Concerning long-chain SFA, significant differences were observed in relation to the species. In particular, *L. luteus* showed the highest content of these long-chain fatty acids. Such findings are interesting from a nutritional point of view because oils with high levels of long-chain SFA were reported to be difficult to digest in both humans and animals (Akpinar et al., 2001).

Among the MUFAs, the content of the erucic acid – that is considered an antiquality factor for animal and human metabolisms – in our samples was lower than that observed by Bhardwai et al. (2004) in white lupin seed variety Lunoble (23.6 g/kg) and variety Lucyanne (27.3 g/kg), by Boschin et al. (2007) in white lupin seed variety Luxe grown in thirteen Italian environments (39–53 g/kg) and by Volek and Marounek (2011) in white lupin seed variety Amiga (36.9 g/kg). Even though the effects of erucic acid on human health are controversial, the government regulation of the European Union limits the levels of erucic acid for human consumption to a maximum of 50 g/kg of the total level of fatty acids in the fat component (Kuhnt et al., 2012). Moreover, a content of erucic acid up to 30 g/kg is not considered detrimental to human health (Joint FAO/WHO, 2015). In this regard, the values obtained in this study are under the maximum level fixed for this acid. The FAO/WHO has developed for the rapeseed oil the definition of 'erucic acid-free oil' when the content is lower than 10 g/kg and of 'oil with a low erucic acid content' when the content is lower than 20 g/kg (Joint FAO/WHO, 2015). In this view, considering the mean values of the lupin species, *L. albus* and *L. luteus* may be defined as 'oil with a low erucic acid content', whereas *L. angustifolius* may be considered 'erucic acid-free oil'.

Data regarding PUFAs are in accordance with those reported by Uzun et al. (2007) in *L. albus* seeds for linoleic acid (203 g/kg) and linolenic acid (92 g/kg). The high content of essential fatty acids (C18:2n6 and C18:3n3) found in lupin oil is typical of many legumes (Bağci, 2006) and suggests that this legume seed could be used in the feeding mixture for farm animals to improve the nutritional quality of their products.

The n-3/n-6 PUFA ratio was quite different between the three lupin species: the one of *L. albus* is certainly comparable to that of canola oil (0.45), while *L. luteus* and *L. angustifolius* showed a ratio more similar to that of most vegetable oils, for example olive oil (0.13), soya bean oil (0.15) and walnut oil (0.20) (Belitz and Grosch, 1999). Moreover, the n-3/n-6 PUFA ratio was in line with Boschin et al.'s (2007) observations in white lupin seed variety Luxe (0.45–0.63). The studies on the relationship between n-3/n-6 PUFA ratio and the pathogenesis of many diseases indicate that the optimal ratio may vary with the disease or condition under consideration; this is consistent with the fact that many diseases are multigenic and multifactorial (Calabrò et al., 2015). The n-3/n-6 profiles of lupin seeds were in the range 1:1 and 1:4, which is considered optimal for human and animal nutrition (Simopoulos, 2003).

Overall, variations among the lupin species and, in some cases, within these, of the genotype on the content of fatty acids may also exert a key role in determining the best lupin variety for animal feeding. In fact, as aforementioned, the higher values of PUFA/SFA, UFA/SFA and n-6 PUFA and the lower values of SFA, TI and AI confirm the higher nutritive value of the lipid fraction of *L. luteus* compared to that of *L. albus* and *L. angustifolius*, strengthening the reliability of the quality indices proposed by Ulbricht and Southgate (1991) in order to evaluate the different nutritional aspects of the various fatty acids. These authors suggested that the AI and TI, strictly related to the entire fatty acid profile, might better characterize the health benefits of a vegetable or animal food than a simple approach based on fatty acid classes or fatty acid ratios (Fehily et al., 1994).

Concerning the positive effect in the livestock food chain 'from feed to food', little information on the effects of lupin seeds on fatty acid composition of pork can be found in the literature (Van Nevel et al., 2000). Zraly et al. (2007) obtained a significant lower content of palmitic acid and a significant increase in oleic acid in meat of pigs fed with lupin (20% inclusion of white lupin seeds, variety Amiga); moreover, the concentration of n-3 PUFA increased significantly as well as the alpha-linolenic acid content. Froidmont et al. (2005) comparing diets with 15% of soya bean meal or 20% of lupin seeds (*L. albus* variety Arès) in Pietrain × Landrace pigs during the growing–finishing period found any influence of the diet on the fatty acid profile of the meat. However, the back fat of pigs receiving the lupin-based diets contained more MUFAs and fewer PUFAs than pigs given soya bean meal diet. As suggested by Mourot (2001), this

modification reflected the composition of dietary fat; the fat of lupin seeds is characterized by a high concentration of oleic acid, while the soya fat used in the diet was rich in linoleic acid. Hence, lupin possess significant advantages other than being a nutrient source for finisher pigs, showing itself to be a potential nutritional management tool to enhance effectively the nutritional value of pork.

In vitro fermentation characteristics

Overall, the *in vitro* fermentation characteristics, end products and kinetics indicated an efficient substrate utilization by the micro-organisms present in the *inocula* apparently not negatively influenced by the alkaloid content. However, no comparisons can be made with reference to this because most of the *in vitro* data carried out with pig *inocula* utilize carbohydrate rather than protein source. Moreover, the methods are not always superimposable in terms of incubation time (24, 48, 96 h) and source of *inocula* (faecal vs. caecal content).

As reported by Menke and Steingass (1988) for legume seeds, the high OM degradability may be explained mainly by protein and starch content; otherwise, gas production only explains a small part of the total dOM variation, which is quite low when compared with the values given in other concentrate feeds. Regarding the fermentation kinetics, all lupin samples showed a very fast process in terms of hours to reach maximum rate but not consistent in terms of values of maximum rate achieved.

The SCFAs are the main end products of the microbial fermentation activity at the expense of carbohydrates in the pig intestine. One of the most significant SCFA properties is the trophic effect on the intestinal epithelium, maintaining the mucosal defence barrier against invading organisms. In particular, butyric acid seems to be the most effective; moreover, it is also an important energy source for the colonic epithelium and regulates cell growth and differentiation (Salminen et al., 1998).

Probably, the presence of raffinose family oligosaccharides (RFOs) in lupin seeds partially affected the fermentation process during the incubation *in vitro*. As reported by Karnpanit et al. (2016), lupin represents a good source of RFOs, which ranged from 7.6 to 16.8 g per 100 g DM. Sources of RFOs could help to create a stable environment within the GIT reducing the incidence of post-weaning diarrhoea because they are easily fermentable by the microflora in the large intestine. Thomsen et al. (2007) observed that the inclusion of sweet lupin completely protected against the development of swine dysentery stimulating the growth or metabolism of special bacterial species (Williams et al., 2001) and may possess prebiotic effect.

Regarding ammonia production, that is a direct end product of protein metabolism; at the end of the *in vitro* incubation, it showed quite high values. This result could be attributed to the high protein content in the leguminous seeds.

The pH values registered after 48 h of fermentation of all tested lupins are consistent with pH values measured in pig large intestines in physiological condition (Bach Knudsen and Hansen, 1991). This result indicated a regular fermentation pattern thanks to the effective action of the buffer solution.

The analysis of correlations showed that some parameters of chemical composition and fatty acid profile significantly ($p < 0.01$) affected the fermentation process. In particular, CP and EE showed similar effects on the fermentation parameters: positive correlations (Pearson's *r*) were found for the dOM (CP: 0.75; EE: 0.74) and negative (CP: −0.69; EE: −0.58) for the potential gas production (A). These data can have different explanations: a high CP content in the incubated substrates increases the degradability (Abreu and Bruno-Soares, 1998) but does not contribute to the gas production, whereas lipids are able to depress the activity of cellulolytic micro-organisms. As regards the kinetics, these nutrients improved the fermentation, in terms of time and rate. On the contrary, as expected, the structural carbohydrates negatively influenced OM degradability (ADF: −0.50; TDF: −0.46) and fermentation rate (ADF: −0.83; TDF: −0.69). The dietary fibre was negatively correlated with most of the fermentation parameters (dOM, R_{max}, SCFAs); probably, these results are in part due to the predominance of insoluble fibre fraction (as average 87.7% of TDF). Instead, lignin content represented a small amount in all the *Lupinus* spp. and consequently did not affect negatively any fermentation parameters, as is reported by Abreu and Bruno-Soares (1998).

Also, the fatty acids showed some significant ($p < 0.01$) correlation with the *in vitro* fermentation, even though SFA influenced diverse parameters compared to MUFA and PUFA (significant Pearson's r for A and B in SFA and for dOM, T_{max}, R_{max}, propionic, SCFAs, in MUFA and PUFA). Moreover, MUFA affected positively these parameters, whereas PUFA negatively influenced them, probably due to the different structure of these compounds.

Conclusion

In conclusion, comparing the three lupin species, the six varieties of *L. albus* showed interesting nutritional and dietetic characteristics: high protein level and low structural carbohydrate content. The lipid fraction, rather elevated, was well represented by MUFA, whereas SFAs are less and the n-3/n-6 ratio is the most favourable. Moreover, in this variety, the tendency to increase the rate of gas production during the early stages of fermentation suggests that the high presence of alkaloids did not affect the *in vitro* degradability, production of SCFAs and fermentation process, probably due to their concentration and/or water solubility.

In particular, the two varieties Multitalia and Lublanc showed the most interesting characteristics in terms of nutritional value associated with fatty acid composition, degradability and fermentation kinetics, even if their alkaloid content was the highest among the varieties of all lupin species. Instead, it seems interesting to highlight the variety Lutteur that showed good characteristics, high SDF and butyric acid production, representing also a potential prebiotic and may affect the intestinal microbiota of pigs in order to achieve beneficial health effects for the animal.

The three varieties belonging to *L. luteus* showed intermediate chemical composition, but the most favourable dietetic aspects, in terms of PUFA content, n-6 PUFA series, UFA/SFA and PUFA/SFA ratios and atherogenic and thrombogenic indices and very low alkaloid content. The *in vitro* fermentation characteristics of this species resulted slightly low compared to the other two species.

Finally, the three varieties of *L. angustifolius* appeared as the least interesting from the nutritional and dietetic point of view, showing a low protein and fat content and a high cell wall level, high SFA amount and the least favourable nutritional indices, even though only the varieties of *L. angustifolius* may be considered 'erucic acid-free oil'. *The in vitro* fermentation patterns of *L. angustifolius* showed intermediate values compared to the other two species.

From a methodological point of view, the gas production technique is confirmed to be a useful tool for studying the utilization of feedstuffs in the pig large intestine. On the whole, lupin, due to its valuable nutritional aspect, may represent a very interesting dietetic alternative to soya bean in pig feeding. However, according to the variability found, the choice of the variety is a crucial aspect to be considered. Moreover, for its practical application in the formulation of pig diets, further investigations are required.

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Conflict of interest

The authors have declared no conflict of interest.

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