Spanish Journal of Agricultural Research
14(1), e0901, 14 pages (2016)
eISSN: 2171-9292
http://dx.doi.org/10.5424/sjar/2016141-7450
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)

RESEARCH ARTICLE

OPEN ACCESS

Biochemical characterization of legume seeds as ingredients in animal feed

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Abstract

The current European protein deficit is estimated as high as 70% of present needs. Because of the high protein content of their seeds, grain legumes are attractive candidates for lowering the deficiency in plant protein production. The objective of this work was to identify new sources of vegetable protein that would reduce our high dependence of soy, the main source of protein in the manufacture of feedstuffs. To achieve this goal, we determined the proximate composition, the bioactive components, as well as the antinutritional factors present in the studied seeds. In general, the protein, fat and carbohydrates content of legume seeds studied were within the limits found in the literature. The bioactive compounds detected in all the seeds were α-galactosides, myoinositol phosphates, protease inhibitors and phenols. IP_6 (phytic acid) was the main inositol phosphate form in all the samples. The highest protease inhibitors content was detected in both *Lathyrus cicera* cultivars. *Vicia ervilia* and *L. cicera* cultivars showed low haemagglutinating activity (20.4 HU/g). The γ-glutamyl-S-ethenyl-cysteine content in *Vicia narbonensis* was around 16.0 mg/g. Both *L. cicera* varieties presented similar β-N-oxalyl-L-α, β-diaminopropionic acid content (0.80 mg/g). The two *V. ervilia* varieties showed high canavanine concentration (1.93-5.28 mg/g). Vicine was only detected in *V. narbonensis* cultivars (0.3 mg/g). The biochemical characterization carried out in this study allows us to know the limits of inclusion of these minor crop seeds in feed formulations in order to replace the soybean.

Additional key words: legumes; bioactive compounds; feedstuffs; *Cicer*; *Vicia*; *Lathyrus*

Abbreviations used: ANF (anti-nutritional factors); CIU (chymotrypsin inhibitor unit); GEC (γ-glutamyl-S-ethenyl-cysteine); HU (haemagglutinating unit); IP (inositol phosphates); ORAC (oxygen-radical absorbance capacity); PCAF (sodium amminepentacyanoferrate (II) hydrat); SBM (soybean meal); SCFA (short-chain fatty acids); TIA (trypsin inhibitor activity); TIU (trypsin inhibitor unit); β-ODAP (β-N-oxalyl-L-α, β-diaminopropionic acid)

Citation: Martín-Pedrosa, M.; Varela, A.; Guillamon, E.; Cabellos, B.; Burbano, C.; Gomez-Fernandez, J.; de Mercado, E.; Gomez-Izquierdo, E.; Cuadrado, C.; Muzquiz, M. (2016). Biochemical characterization of legume seeds as ingredients in animal feed. Spanish Journal of Agricultural Research, Volume 14, Issue 1, e0901. http://dx.doi.org/10.5424/sjar/2016141-7450.

Received: 29 Jan 2015. **Accepted**: 01 Feb 2016

Funding: INIA-COPISO Contract (CON08-078) and the Spanish CDTI Project (IDI-20100284).

Competing interests: The authors have declared that no competing interests exist.

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Introduction

Legume crops are relevant for the European agriculture and nutrition, both for food and feed being a major source of plant protein. Legumes contribute to a sustainable improvement of the environment when they are included in agricultural rotations due to their effect on biological nitrogen fixation in the soil, the control of weeds and the services given to other components of the agro-ecosystems.

However, growing legumes in Europe has been declining in the past 40 years for reasons including: price competition of feedstock proteins produced mainly in North and South America; the increased use of chemical fertilizers in crop production; the availability of cheap sources of animal protein (fish and meat-beef, pork and poultry), and the simplification of agricultural systems. In fact, during the last decades the cultivation of grain legumes in Europe, as well as their use, fell sharply: in 2010 the total production of pulses in Europe was 39% of that obtained in 1990 (FAO Stat: http://faostat3.fao.org/home/index.html). As a consequence, Europe presently needs to import most of the

plant proteins for human consumption and animal use with negative repercussions on the European trade balance. The current European protein deficit (and relative import values) is estimated as high as 70% of present needs, which puts Europe in a serious competitive disadvantage towards other countries.

The demand for plant proteins for human nutrition has increased tremendously for the last years in Europe due to: i) demographic growth and urbanization, ii) the limited land areas which can be used for production of food crops, whereas farming systems are switching to specialized cereal production (for market competitiveness) even if not sustainable farming, and iii) decrease in animal protein production due to shortage of irrigation and/or rainfall water, especially in Mediterranean areas.

Because of the high protein content of their seeds, grain legumes are attractive candidates for lowering the deficiency in plant protein production, however, low improvement in farming practices has been achieved over the last years to enhance production of traditionally grown grain legumes such as faba bean, common bean or lupin. Special attention has to be paid to the limiting factors affecting legume yield, in order to obtain more regular production. Even though these constraints have become structural in the European environment, very limited research and development efforts have been devoted to strategies to improve grain legume production under stress conditions to contribute to the development of sustainable agriculture in the European Area.

Most worrying is the fact that throughout Europe there has occurred a progressive loss of the know-how to grow legumes in rotations, their proper harvesting, storing and preparation of the seed for further reproduction and conversion skill is progressively being lost. In addition, the use of legumes in the diet and knowledge on how to use legumes in food preparations are decreasing, although a wider consumption of plant proteins than of animal proteins continues to be recommended by physicians.

Many documents of national and international relevance and the European policies and plans refer to the above mentioned problems such as: the United Nations Millennium Declaration in 2000 (A/RES 55/2: http://www.un.org/millennium/declaration/ares552e.htm), the United Nations World Summit Outcome (2005); the European Union Research and Innovation Programme 2014-2020 (Horizon 2020: http://ec.europa.eu/programmes/horizon2020); European Commission documents: COM 279 in 2007 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=COM:2007:0279:FIN:EN:PDF), COM 672 in 2010 (http://ec.europa.eu/agriculture/cap-post-2013/communication/com2010-672_en.pdf), and SEC 1153 in 2011 (http://ec.europa.eu/agriculture/policy-perspectives/impact-assessment/cap-towards-2020/report/annex3a-d_en.pdf).

To reverse the present situation, actions have to be immediately taken since a wider use of legumes in crop rotation enables significant benefits in economic, environmental and climate change areas. Approaches aimed to the improvement and exploitation of seed nutritional and technological qualities are needed and expected to drive consumers and farmers towards new, diverse, healthier and more sustainable choices (Muzquiz *et al.*, 2012). However, the use of grain legumes in animal nutrition has been limited in practice due to partially high concentrations of secondary plant metabolites, also referred to as antinutritional factors (ANFs), including condensed tannins, protease inhibitors, lectins, pyrimidine glycosides, etc. Possible negative effects of these secondary plant metabolites include, for example, feed refusals (tannins, alkaloids), reduced nutrient digestibilities (tannins, protease inhibitors, lectins) or even toxic effects (β-N-oxalyl-L-α, β-diaminopropionic acid or β-ODAP). Furthermore, a high proportion of α-galactosides as a part of the carbohydrate fraction in some grain legumes may cause detrimental effects in animals, such as excessive fermentation, flatulence and diarrhoea. In spite of the progress in plant breeding, there are still only few examples of grain legumes with low levels of these ANFs (Jezierny *et al.,* 2010). To solve these problems, efforts by assembled European scientists and experts with a long-time experience in legume research are required to reinforce the cultivation of legumes in Europe for both food and feed. Also, an integrated structure designed to strengthen the competitiveness of European food producers taking into account small and medium enterprises interests and needs would be of great help in the worldwide context.

The objective of this work was to identify new sources of vegetable protein that would reduce our high dependence upon soybean (*Glycine max* L.), the main source of protein in the manufacture of feedstuffs. Since, the knowledge about the composition of some minor crops seeds will provide information for establishing their limits of inclusion in feed formulations and will allow the replacement of soybean and their derivatives.

Material and methods

Plant materials

The seed materials were part of the Spanish Center for Technological and Industrial Development (CDTI)

Project (IDI-20100284) in collaboration with COPISO (Agricultural Cooperative Industry of Soria, Spain). Cultivars of *Lathyrus cicera* (13), *Cicer arietinum* (24), *Vicia narbonensis* (16) and *Vicia ervilia* (24) were grown and harvested by ITACyL (Agricultural Technology Institute of Castilla-León, Spain) in two consecutive years (2010/2011 and 2011/2012, autumn sowing) at five different locations of Castilla y León, a central/northwest region of Spain: Finca Zamadueñas (41º 42' N, 4º 41' W) and Trigueros del Valle (41º 49' N, 4º 39' W), both in the province of Valladolid; Esteras de Lubia (41º 43' N, 2º 10' W) and Barca (41º 27' N, 2º 37' W), both in Soria and Cordovilla La Real (42º 4' N, 4º 15' W) in Palencia.

In the trial fields (12 m^2, n=3, randomized), environmental data, phenology, pests and diseases and yields were evaluated and nine cultivars were finally selected: 2 of *Lathyrus cicera* (ZL-41, ZL-02), 3 of *Cicer arietinum* (Duratón, ELF, Tizón), 2 of *Vicia narbonensis* (ZU-154, ICARDA-2470) and 2 of *Vicia ervilia* (Villanueva, Taranto). For nutritional/antinutritional evaluation, the same amount of seeds from each cultivar and location were mixed and randomly sampled. Seeds from each sampled cultivar were individually ground in a Cyclotec 1093 Sample Mill (Tecator, Hoganas, Sweden), passed through a 1 mm sieve and stored at -20°C until use. In addition, a defatted soya cake, soybean meal (SBM) FEDNA47 type (47% crude protein) (FEDNA: Spanish Foundation for the Development of Animal Nutrition), a common raw material in feed formulations, was also evaluated to be used as control, passed through a 1 mm sieve and stored in the same conditions. Four subsamples of each sample type were taken for further analysis.

Chemical analyses

Proximate analysis

Four subsamples of each variety were taken for further analysis. Two replicates per subsample were analysed for each component. The ash, protein and fat contents of the samples were determined according to official AOAC procedures (2000). The ash content was calculated from the weight remaining after heating the sample at 550ºC. The protein content was determined using the Kjeldahl method and applying a nitrogen-to-protein conversion factor of 5.71. Soxhlet extraction was used to determine the fat content. Total carbohydrates were calculated by difference.

All chemicals used in this work were of analytical grade.

Soluble sugars

The concentration of soluble sugars in seed fractions was determined by HPLC following a modification of the Muzquiz *et al.* (1992) method. Samples (0.1 g) were homogenized in aqueous ethanol (50% v/v, 5 mL) for 1 min using an Ultraturrax homogenizer (T25 basic; IKA, Königswinter, Germany). The mixture was centrifuged for 5 min at 12,100 × g. The supernatant was decanted and the procedure repeated twice. The combined supernatants were passed through Sep-Pak C_{18} cartridges (500 mg, Waters, Milford, MA, USA) and the column was washed with 3 mL aqueous ethanol (50% v/v). The combined extracts and washings were collected and evaporated to dryness. The residue was re-dissolved in 1 mL of double deionised water, and centrifuged for 8 min at 12,100 × g. Before injection samples were filtered through a 0.45 µm Millipore (Carringtwohill, Ireland) membrane. Two replicates of each sample were analyzed. Aliquots of 20 µL were injected into a HPLC system (Beckman System Gold Instrument, Los Angeles, CA, USA) equipped with a refractive index detector. A Spherisorb-5-NH_2 column (250 × 4.6 mm i.d., Waters, Milford, MA, USA) equilibrated with acetonitrile/water 60:40 (v/v) was used with a flow rate of 1 mL/min.

Individual sugars were quantified by comparison with external standards of pure sucrose, raffinose, ciceritol and stachyose (Sigma, St. Louis, MO, USA). Verbascose was purified and kindly supplied by Dr. A. I. Piotrowicz-Cieslak (Olsztyn-Kortowo, Poland). Calibration curves were constructed for all standard sugar solutions. A linear response was evident in the range (0-5 mg/mL) with a correlation coefficient of 0.99.

Inositol phosphates

Individual inositol tri-, tetra-, penta-, hexaphosphates (IP_3-IP_6) were extracted according to Burbano *et al.* (1995a) with some modifications and determined by the Lehrfeld (1994) method. A 0.5 g sample was extracted with 5 mL of 0.5 M HCl for 1 min using an Ultraturrax homogenizer (T25 basic; IKA, Königswinter, Germany). The extract (2.5 mL) was diluted with 25 mL of deionised water and placed onto a SAX column (Varian, Lake Forest, CA, USA). The column was washed with 2 mL of deionised water, and the inositol phosphates eluted with 2 mL of 2 M HCl. The eluate was evaporated to dryness and the residue dissolved in a buffer solution. The solution was centrifuged at 12,100 × g for 6 min to remove any suspended material prior to injection into the HPLC (Beckman System Gold Instrument). The column consisted of a macroporous polymer PRP-1 (150 × 4.1 mm i.d., 5 µm,

Hamilton, Reno, NV, USA) which was maintained at 45ºC, with a flow rate of 1 mL/min.

Individual inositol phosphates were quantified by comparison with external standard of IP_6 (Sigma).

Protease inhibitors

Trypsin inhibitor activity. Trypsin inhibitor units (TIU) were defined according to Welham & Domoney (2000). Trypsin inhibitor activity was determined by using α-N-benzoyl-DL-arginine-p-nitroanilidehydrochloride (Sigma) as the trypsin substrate. TIU/mg flour were calculated from the absorbance read at 410 nm against a reagent blank. One unit of TIU was defined as that which gave a reduction in A_{410nm} of 0.01 relative to trypsin control reactions, using a 10 mL assay volume.

Chymotrypsin inhibitor activity. Chymotrypsin inhibitor units (CIU) were defined according to the assay described by Shate and Salunkle (1981) with some modifications. The assay mixture consisted of inhibitor in assay buffer, 100 mM Tris-HCl containing 20 mM $CaCl_2$ at pH 7.8. Chymotrypsin inhibitor activity was determined at 30ºC using Benzoyl-L-tyrosine ethyl ester as chymotrypsin standard (Sigma). Absorbance at 256 nm was recorded 7 minutes after the substrate addition. One chymotrypsin inhibitor unit was defined as the increase by 0.01 absorbance unit at 256 nm of the reaction mixture.

Haemagglutinating activity

Samples were extracted with 0.1 M PBS (pH 7.4) and the haemagglutinating activity was estimated in the PBS extracts by a serial dilution procedure using native and trypsin treated rat blood cells (Grant, 1991). The amount of material (mg) causing 50% agglutinated erythrocytes was defined as that, which contained 1 haemagglutinating unit (HU). For comparison, values were expressed as HU/g seed meal. *Phaseolus vulgaris* cvs. Processor and Pinto were included in each assay as positive and negative controls, respectively. Soybean lectin from Sigma diluted in PBS was also used as standard.

Phenolic compounds

For extracting polyphenolic compounds, the flours (2.5 g) were macerated three times with a solution of methanol-HCl ($1^0/_{000}$)/water (80:20 v/v) in orbital shaker, at room temperature (Dueñas *et al.,* 2005). The combined macerates were brought to a fixed volume (15 mL) and were used for the quantification of total phenolic compounds (Oomah *et al.,* 2005) and the antioxidant activity by the Oxygen-Radical Absorbance Capacity (ORAC) method (Dávalos *et al.,* 2004).

γ-glutamyl-S-ethenyl-cysteine (GEC)

Ground seeds (500 mg) were extracted twice by stirring in 5 mL of ethanol/water (70:30 v/v) for 1 h. The slurries were centrifuged at 5000 × g for 30 min, and the resulting supernatants were recovered and taken to a volume of 10 mL (Sanchez-Vioque *et al.,* 2011).

Colorimetric determination was performed according to Njaa (1980) with modifications (Sanchez-Vioque *et al.,* 2011). Aliquots (20 µL) of the ethanol/water extracts were deposited in test tubes and kept in an oven at 40 ºC until dryness. After cooling the samples at room temperature, 2 mL of 0.15 M phosphate buffer (pH 7.0) and 1 mL of iodoplatinate reagent were added. Samples were left in the dark for 2 h, and the absorbance was measured at 505 nm against phosphate buffer. A calibration curve was made with standard GEC in the range of 5.3-33 µg (10-59.78 µmol/L) (Sanchez-Vioque *et al.,* 2011).

Canavanine

The L-canavanine content was determined according to Cacho *et al.* (1989) and Sanchez-Vioque *et al.* (2008). Samples (500 mg) were extracted twice by Ultraturrax (T25 basic; IKA, Königswinter, Germany) with 5 mL of 0.1 N HCl for 2 min. The slurries were centrifuged at 10000 × g for 20 min and the supernatants recovered and the volumes completed to 10 mL in volumetric flasks. About 300 µL of each extract were added with 300 µL of distilled water, 1.950 mL of 0.2 M sodium phosphate pH 7.0, 300 µL of 1% potassium peroxodisulphate and 150 µL of 1% sodium amminepentacyanoferrate (II) (PCAF) hydrate. This mixture was incubated at room temperature in the dark for 15 min and the absorbance measured at 520 nm. The amount of L-canavanine was calculated from a calibrating curve (0.005-0.08 mg/mL) made with the pure product. The PCAF reagent was prepared according Cacho *et al.* (1989).

Vicine and convicine

The concentration of vicine and convicine in the samples of *Vicia narbonensis* and *Vicia ervilia* was determined by duplicate using the method of Burbano *et al.* (1995b) based on that of Marquardt & Fröhlich (1981). The compounds were extracted from 0.05 g flour with 5 mL of 0.83 M perchloric acid for 1 min

using an Ultraturrax homogenizer (T25 basic, IKA, Germany) at 4 ºC. The extract was centrifuged and filtered through a 0.45µm filter (Millipore, Ireland) prior to injection into the HPLC chromatograph (Beckman System Gold) equipped with a UV-visible detector fixed at 280 nm. Chromatographic conditions were a reversed-phase C_{18} column (Spherisorb ODS, 250 × 4.6 mm, 5 µm, Waters) with ammonium phosphate buffer (0.05 M, pH 2.0) as mobile phase at 1.0 mL/min. Standards of pure vicine and convicine were obtained from Dr R. Marquardt (University of Manitoba, Canada). Calibration curves were drawn for the two compounds. There was a linear response in the range 8.6-310.0 mg/L for vicine and 3.7-134.0 mg/L for convicine.

Statistical analysis

To establish the statistical significance of differences (p<0.05), a one-way ANOVA was applied to the obtained analytical data, as well as Duncan's multiple range test. Statgraphics Plus 5.1 computer package (Graphics Software System, Rockville, MD, USA) was used, while Pearson correlation test was conducted to determine the correlation between variables. Significant levels were defined using $p \leq 0.01$.

Results

The ash, protein, fat and total carbohydrates content of the legume seeds here studied are presented in Table 1. Significant differences in the proximate composition of all the samples were determined. All are regarded as valuable alternative raw material in feed formulations. However, legume seeds contain a great number of bioactive compounds that vary considerably in their biochemistry, as indicated below.

Soluble sugars

Table 2 summarizes the soluble sugars content of sucrose, maltose, ciceritol, galactinol, galactopinitol and α-galactosides in the different legumes. All samples contained different amounts of sucrose, ciceritol, raffinose, stachyose and verbascose. In *L. cicera* seeds maltose, galactopinitol and verbascose were not detected. Verbascose was only detected in the cultivars of *V. narbonensis* and *V. ervilia* and maltose, galactinol and galactopinitol were not detected in *V. narbonensis*. The legumes seeds differ significantly in the total α-galactosides or raffinose oligosaccharides (raffinose, stachyose and verbascose) content and ranged from 7.31 mg/g in *V. ervilia* to 52.95 mg/g in *V. narbonensis*.

Inositol phosphates

The inositol phosphates content is presented in Table 3. All the samples contained mainly IP_6 (phytic acid) and different concentrations of IP_5 and IP_3 except that of *C. arietinum* var. Tizón that did not contain IP_3. IP_4 was only detected in SBM and *V. narbonensis* Icarda-2470 seeds. The samples differed significantly in the total inositol phosphates content.

Table 1. Ash, protein, fat and carbohydrates content (mg/g) in different crop seeds and soybean meal. Mean values ± standard error; n=8.

Samples	Ash	Protein	Fat	Total carbohydrates
SBM 47	73.5 ± 0.84^{a}	470.0 ± 3.70^{a}	18.0 ± 0.74^{d}	356.2 ± 5.76^{a}
L. cicera ZL-41	30.5 ± 0.35^{e}	268.1 ± 4.23^{d}	$7.6 \pm 0.31^{a,b}$	$616.8 \pm 10.00^{c,d}$
L. cicera ZL-02	63.9 ± 0.73^{b}	249.3 ± 0.53^{e}	6.6 ± 0.27^{a}	604.3 ± 9.77^{c}
C. arietinum Duratón	27.1 ± 0.31^{g}	228.2 ± 0.42^{g}	50.5 ± 2.07^{f}	$613.3 \pm 9.90^{c,d}$
C. arietinum ELF	$27.8 \pm 0.35^{f,g}$	218.7 ± 0.84^{h}	40.9 ± 1.67^{e}	641.2 ± 10.37^{d}
C. arietinum Tizón	27.5 ± 0.35^{g}	212.1 ± 1.37^{i}	42.1 ± 1.72^{e}	$626.6 \pm 10.10^{c,d}$
V. narbonensis ZU-154	33.9 ± 0.39^{d}	272.1 ± 3.70^{c}	$10.1 \pm 0.41^{b,c}$	534.5 ± 8.65^{b}
V. narbonensis Icarda-2470	$35.2 \pm 0.^{40c,d}$	282.6 ± 0.85^{b}	15.5 ± 0.63^{d}	526.0 ± 8.51^{b}
V. ervilia Villanueva	$29.3 \pm 0.33^{e,f}$	237.3 ± 0.85^{f}	15.8 ± 0.65^{d}	610.6 ± 9.88^{c}
V. ervilia Taranto	35.6 ± 0.41^{c}	251.9 ± 1.58^{e}	11.2 ± 0.46^{c}	605.2 ± 9.70^{c}

SBM: soybean meal. Mean values in the same column followed by a different superscript letter are significantly (p<0.05) different.

Table 2. Content (mg/g) of different soluble sugars in different minor crop seeds and soybean meal. Mean values ± standard error; n=8.

Samples	Sucrose	Maltose	Ciceritol	Galactinol	Galactopinitol	Raffinose	Stachyose	Verbascose
SBM 47	53.48 ± 0.66^{a}	3.60 ± 0.43	0.86 ± 0.10	ND	ND	13.54 ± 0.41	43.99 ± 0.12	ND
L. cicera ZL-41	6.08 ± 0.27^{j}	ND	2.21 ± 0.13	0.45 ± 0.05	ND	6.09 ± 0.16	30.73 ± 0.12	ND
L. cicera ZL-02	7.58 ± 0.09^{i}	ND	0.93 ± 0.08	0.80 ± 0.12	ND	7.11 ± 0.11	36.15 ± 0.41	ND
C. arietinum Duratón	13.85 ± 0.05^{d}	2.29 ± 0.11	29.65 ± 0.33	0.76 ± 0.05	2.33 ± 0.13	5.59 ± 0.19	18.26 ± 0.33	ND
C. arietinum ELF	12.04 ± 0.22^{e}	1.30 ± 0.11	22.83 ± 0.33	0.65 ± 0.15	3.49 ± 0.13	4.51 ± 0.11	16.73 ± 0.73	ND
C. arietinum Tizón	10.28 ± 0.11^{f}	1.59 ± 0.09	26.05 ± 0.25	0.43 ± 0.03	3.71 ± 0.18	5.31 ± 0.07	19.64 ± 0.19	ND
V. narbonensis ZU-154	8.53 ± 0.19^{h}	ND	7.51 ± 0.35	ND	ND	4.72 ± 0.08	15.89 ± 0.51	27.90 ± 0.34
V. narbonensis Icarda-2470	9.09 ± 0.10^{g}	ND	7.49 ± 0.07	ND	ND	5.47 ± 0.06	17.79 ± 0.17	29.69 ± 0.44
V. ervilia Villanueva	18.73 ± 0.13^{b}	3.41 ± 0.18	10.39 ± 0.10	ND	13.93 ± 0.27	ND	1.50 ± 0.27	5.81 ± 0.14
V. ervilia Taranto	18.10 ± 0.59^{c}	4.95 ± 0.21	10.72 ± 0.27	ND	12.68 ± 0.49	2.71 ± 0.19	0.45 ± 0.08	7.83 ± 0.12

SBM: soybean meal. Mean values in the same column followed by a different superscript letter are significantly (p<0.05) different. ND: not detected.

Table 3. Inositol phosphates (IP3: inositol trisphosphate; IP4: inositol tetrakisphosphate; IP5: inositol pentakisphosphate; IP6: inositol hexakisphosphate) content (mg/g) in different minor crop seeds and soybean meal. Mean values ± standard error; n=8.

Samples	IP3	IP4	IP5	IP6	Total IP
SBM 47	0.20 ± 0.02	0.61 ± 0.18	2.03 ± 0.21	12.69 ± 0.29	15.54 ± 0.15^{a}
L. cicera ZL-41	0.10 ± 0.01	ND	0.49 ± 0.02	8.24 ± 0.32	8.83 ± 0.35^{b}
L. cicera ZL-02	0.07 ± 0.01	ND	0.44 ± 0.01	5.27 ± 0.11	5.79 ± 0.11^{g}
C. arietinum Duratón	0.08 ± 0.00	ND	0.46 ± 0.04	6.36 ± 0.08	6.91 ± 0.10^{d,e}
C. arietinum ELF	0.07 ± 0.00	ND	0.34 ± 0.02	5.30 ± 0.06	5.71 ± 0.07^{g}
C. arietinum Tizón	ND	ND	0.36 ± 0.02	5.73 ± 0.11	6.10 ± 0.09^{f}
V. narbonensis ZU-154	0.06 ± 0.01	ND	0.34 ± 0.01	5.46 ± 0.13	5.84 ± 0.14^{d}
V. narbonensis Icarda-2470	0.09 ± 0.01	0.10 ± 0.00	0.43 ± 0.02	7.94 ± 0.15	8.51 ± 0.19^{c}
V. ervilia Villanueva	0.19 ± 0.02	ND	0.31 ± 0.02	6.21 ± 0.20	6.71 ± 0.21^{e}
V. ervilia Taranto	0.21 ± 0.00	ND	0.32 ± 0.06	8.11 ± 0.10	8.64 ± 0.04^{b,c}

SBM: soybean meal. Mean values in the same column followed by a different superscript letter are significantly (p<0.05) different. ND: not detected.

Other bioactive compounds and antinutrients content in different minor crop seeds and soybean meal

Table 4 summarizes the content of protein-antinutrients (trypsin and chymotrypsin inhibitors and lectins), phenolic compounds and antioxidant activity, γ-glutamyl-S-ethenyl-cysteine (GEC), L-canavanine and the pyrimidine glycosides vicine and convicine.

Protein-antinutrients

The trypsin inhibitors content in the studied legumes ranged from 6.52 TIU/mg in *V. ervilia* cv.Villanueva to 16.28 TIU/mg in *L. cicera* ZL 41. SBM was subjected to a heat treatment which abolished most of the protease inhibitors activity (Table 4). The highest values of chymotrypsin inhibitors (16.60-20.78 CIU/mg) were found in the samples of *L. cicera* seeds. There were significant differences between species and varieties in the protease inhibitors content.

In this study, an initial evaluation of haemagglutinating activity content in all legume samples was carried out by using the native rat blood cells. As all the samples showed low haemagglutination activity with the native assay, trypsinized rat blood cells were used in order to increase the sensitivity of the assay. The haemagglutinating activity in the different legumes measured by this trypsinized haemagglutination assay is reported in Table 4. The results indicated a low haemagglutinating activity in *V. ervilia* and *L. cicera* cultivars with levels similar to the negative control (*P.*

Table 4. Other bioactive compounds content in different minor crop seeds and soybean meal. Mean values ± standard error; n=8.

Samples	TIU/mg	CIU/mg	Haemagglutinating activity (HU/mg)	Phenolic content (mg catechin equivalent/g)	Antioxidant activity (µmol trolox equivalent/g)	GEC (mg/g)	Canavanine (mg/g)	Vicine (mg/g)	Convicine (mg/g)
SBM 47	0.61 ± 0.00^{a}	3.56 ± 0.06^{a}	63.0 ± 20.8^{a}	13.59 ± 0.008^{a}	45.37 ± 12.47^{a}	NA	NA	NA	NA
L. cicera ZL-41	16.28 ± 0.38^{f}	20.78 ± 0.14^{f}	20.4 ± 0.00^{b}	3.35 ± 0.006^{g}	11.31 ± 4.73^{c}	NA	NA	NA	NA
L. cicera ZL-02	15.14 ± 0.38^{g}	16.60 ± 0.50^{e}	20.4 ± 0.00^{b}	4.06 ± 0.003^{d}	$15.70 \pm 3.53^{b,c}$	NA	NA	NA	NA
C. arietinum Duratón	14.51 ± 0.28^{e}	$10.12 \pm 0.61^{c,d}$	ND	3.12 ± 0.026^{h}	$16.17 \pm 2.17^{b,c}$	NA	NA	NA	NA
C. arietinum ELF	12.60 ± 0.45^{d}	9.74 ± 0.22^{c}	ND	2.70 ± 0.009^{j}	$13.98 \pm 2.11^{b,c}$	NA	NA	NA	NA
C. arietinum Tizón	12.70 ± 0.35^{d}	11.38 ± 0.90^{d}	ND	2.87 ± 0.007^{i}	$18.93 \pm 2.75^{b,c}$	NA	NA	NA	NA
V. narbonensis ZU-154	6.95 ± 0.01^{b}	2.19 ± 0.10^{a}	ND	3.87 ± 0.015^{e}	26.69 ± 0.90^{b}	16.1 ± 0.09	ND	0.3075 ± 0.043	ND
V. narbonensis Icarda-2470	7.01 ± 0.05^{b}	2.63 ± 0.60^{a}	ND	3.67 ± 0.007^{f}	$24.71 \pm 1.40^{b,c}$	16.5 ± 0.93	ND	0.3525 ± 0.015	ND
V. ervilia Villanueva	6.52 ± 0.11^{b}	$8.68 \pm 0.57^{b,c}$	20.4 ± 0.00^{b}	4.59 ± 0.020^{c}	27.78 ± 1.87^{b}	ND	5.28 ± 0.18^{a}	ND	ND
V. ervilia Taranto	7.60 ± 0.08^{c}	8.20 ± 0.12^{b}	$41.6 \pm 0.00^{a,b}$	4.77 ± 0.027^{b}	$20.19 \pm 1.15^{b,c}$	ND	1.93 ± 0.18^{b}	ND	ND

SBM: soybean meal. Mean values in the same column followed by a different superscript letter are significantly ($p<0.05$) different. TIU: Trypsin inhibitors units. CIU: Chymotrypsin inhibitors units. GEC: γ-glutamyl-S-ethenyl- cysteine. HU: Haemagglutination units (mg lectin equivalent/g). NA: not analyzed. ND: not detected.

vulgaris cv. Pinto, 20.4 HU/g). The content measured in both species differed significantly from that in SBM.

Phenolic compounds and antioxidant activity

The total phenolic content and the antioxidant activity values are presented in Table 4. Methanolic extract from all samples exhibited antioxidant capacity, being antioxidant activity of SBM significantly higher (45.37 µmol Trolox equivalent/g of seeds) while ORAC values for the other seeds varied from 11.31 to 27.78 µmol Trolox equivalent/g of seeds. There were significant differences in the total phenolic content among the species and cultivars analysed. In contrast, except for the SBM, there were no significant differences in the antioxidant activities between cultivars and species.

The significant high antioxidant activity of SBM could be related to their high phenolic content. In fact, it is important to notice that a high significant correlation between phenolic contents and antioxidant activities of legume extracts studied were observed ($r=0.877$, $p<0.01$).

γ-glutamyl-S-ethenyl-cysteine (GEC)

This compound is specific of narbon vetch (*V. narbonensis*) and does not appear in SBM and other studied legumes. Table 4 shows its content in the two *V. narbonensis* cultivars, being 16.5 mg/g in ICARDA-2470 and 16.1 mg/g in ZU-154.

L-canavanine

Table 4 shows the significant differences in the canavanine concentration in the two cultivars of *V. ervilia* studied: Villanueva (5.28 mg/g) and Taranto (1.93 mg/g).

Vicine and convicine

The pyrimidine glycosides, vicine and convicine, are compounds present in the genus *Vicia*. *V. narbonensis* cultivars were the only analyzed seeds (Table 4) that contained vicine (0.31 and 0.35 mg/g in ZU-154 and Icarda-2470, respectively).

Discussion

In general, according to the literature, grain legume seeds are characterized by having a relatively high protein (200-300 mg/g) and carbohydrate (500-650 mg/g) contents. The fat content is not usually greater that about 50 mg/g. The exceptions are soybean and the lupin species, which have higher protein contents (350-450 mg/g) and a lower proportion of carbohydrate (300-400 mg/g) in their seeds. However, the fat content in soybean seeds can reach values higher than 200 mg/g (Hedley, 2001). The results obtained in this work were within the limits found in the literature (Hedley, 2001; Lopez Bellido, 1994) except for the fat content of *L. cicera, V. narbonensis* and *V. ervilia* species which were lower (6.6-15.8 mg/g).

Legume seeds contain bioactive compounds of very different chemical nature such as proteins (protease inhibitors, α-amylases, lectins); glycosides (α-galactosides, vicine and convicine); tannins; saponins; alkaloids, etc. (Muzquiz, 2000; Muzquiz *et al.,* 2012). Hence, methods for their extraction, determination and quantification are very specific (Goyoaga *et al.,* 2008; Pedrosa *et al.,* 2012). They do not appear in all plants, and their physiological effects are diverse (Muzquiz *et al.,* 2012).

Some of these bioactive compounds are important in plant defence mechanisms against predators or environmental conditions. Others are reserve compounds, accumulated in seeds as energy stores in readiness for germination (Roberts & Wink, 1998).

Soluble sugars

The α-galactosides, include the water-soluble, low-molecular weight carbohydrates namely raffinose, stachyose and verbascose. Soybean oligosaccharides represent approximately 4% of the soybean dry matter (DM) and they are not removed or destroyed by the production of SBM. Therefore, in SBM, α-galactosides represent approximately 5-6% but could be as high as 8% DM (Zduńczyk *et al.,* 2010). In the present work, stachyose was the major α-galactoside in soybean, *Lathyrus* and *Cicer* samples, which is similar to that reported by other authors (Aranda *et al.,* 2001; Hedley, 2001). Ciceritol was detected at higher concentrations in the *C. arietinum* samples and these results are within those reported by Muzquiz & Wood (2006) for Desi (21.1-31.0 mg/g) and Kabuli (12.4-27.9 mg/g) types. The highest amount of verbascose was detected in *V. narbonensis* (27.90-29.69 mg/g) seeds.

Carbohydrates are important to the growth and development of the seed, forming the main structural elements and the main translocation and storage compounds. Oligosaccharides and galactosyl cyclitols seem to play an important role in the acquisition of desiccation tolerance of somatic embryos (Fordoñski *et al.,* 2001). However, these oligosaccharides are indigestible in the upper intestinal tract of monogastric animals due to the lack of α-galactosidase enzyme. However, they are easily fermented by the lower gut microflora, resulting in the production of various gases and short-chain fatty acids (SCFAs). Some authors were referring to soybean oligosaccharides as bifidogenic factors which stimulate the growth of beneficial bacteria and others claiming that increased consumption of oligosaccharides may lead to negative effects in the large intestine of mammals, such as flatulence, diarrhoea, and excessive dietary protein decay (Zduńczyk *et al.,* 2010).

Taking into account the amounts of SBM usually incorporated into the diets for monogastric animals, α-galactosides concentrations vary within a broad range of 5 mg/g to over 25 mg/g. The results obtained in experiments with chickens and piglets indicate that the physiological effects of α-galactosides are dependent on the concentrations of these carbohydrates in the diet. Neither too high (23-26 mg/g) nor too low (0.5-1 mg/g) α-galactosides content of diets is recommended. A high content of α-galactosides in the diet is known to enhance fermentation processes within the intestines (increased production of SCFAs) and to increase the hydration of the intestinal contents, thus increasing the risk of diarrhoea. A decrease in α-galactosides levels below 1 mg/g significantly increases the viscosity of the intestinal content and has a negative influence on the development of duodenal structures (Jezierny *et al*., 2010).

These analytical values facilitate establishing threshold inclusion levels of these minor crops in diet formulations, which would increase the amount of available feed resources. This is expected to have a positive impact on farms productivity, and at the same time promote animal health and welfare since the presence of certain amounts of α-galactosides has been reported to improve gut health (Zollitsch, 2007).

Inositol phosphates

Different studies in legumes showed that inositol hexakisphosphate (IP_6) and inositol pentakisphosphate (IP_5) were always the main inositol phosphates forms (Burbano *et al.,* 1995a) present in the seeds. The results obtained in this work were in concordance with these studies. Phytic acid, myo-inositol-(1,2,3,4,5,6) hexakisphosphate, and its salts represent the majority of the phosphorus in legume seeds (Urbano *et al.,* 2000; Kumar *et al.,* 2010). IP_6 and IP_5, have the worst antinutritional effects, as the smaller molecules IP_4 (inositol tetrakisphosphate), IP_3 (inositol trisphosphate), IP_2 (inositol bisphosphate) and IP_1 (inositol monophosphate) have a lower capacity to complex with inorganic cations. Usually, IP_4 and IP_3 were present in lower concentration than IP_6 and IP_5. Pulse grains are a dietary source of minerals, although their bioavailability is considered lower because of the concentration of phytate (Sandberg, 2002). Phytate is formed during maturation of the plant seed and in dormant seeds represents 60-90% of the total phosphate content (Loewus, 2002). Usually, legume based food items contain higher phytate amounts than cereal-based food items (Kumar *et al.,* 2010). Phytic acid has been considered an antinutrient as it binds with other nutrients making them inaccessible to digestion. Higher amounts of phytic acid in the

diet can have a negative effect on mineral balance because of the insoluble complexes it forms with essential minerals (Cu^{2+}, Zn^{2+}, Fe^{3+} and Ca^{2+}) which causes poor mineral bioavailability (Urbano *et al.,* 2000; Konietzny & Greiner, 2003). Phytic acid is able to complex with proteins also, thereby decreasing their solubility. Phytate, therefore, impact on enzyme activity and there is evidence of negative effects on key digestive enzymes, including lipase, α-amylase, pepsin, trypsin and chymotrypsin (Urbano *et al.,* 2000; Greiner & Konietzny, 2006). Phytic acid also appears to bind with starch through phosphate linkages (Lajolo *et al.,* 2004).

In order to exert an effect, either local or systemic, ANFs have to survive at least to some extent the digestive process. According to Rubio *et al.* (2006) intestinal apparent digestibility of phytate was 0% for defatted soybean and autoclaved kidney bean in pigs diets, whereas the values for raw lupin and chickpea diets were 4.1 and 24.5%, respectively. As the monogastric organism contains no or only negligible amounts of endogenous phytase in the stomach and small intestine, it is dependent on plant or microbial phytase to hydrolyse these compounds. It is also known that legume seeds exhibit negligible values of phytase activity. Owing to productive and environmental implications, in the last decades, the use of commercial phytase in pig and poultry diets has been increased to provide phytate hydrolysis, improving the mineral bioavailability and reducing the P excretion (Grases *et al.,* 2006). Knowing the IP content of any given feed or foodstuff is therefore a crucial consideration in order to establish the amounts and type of dietary phytase supplementation.

Protein antinutrients

The most widely studied antinutrient proteins in legumes are the protease inhibitors and the lectins (Lajolo *et al.,* 2004; Pusztai *et al.,* 2004).

Protease inhibitors can have a major impact on seed nutritional value as they inhibit the function of digestive enzymes, such as trypsin and chymotrypsin, by competitive binding. In our previous work (Guillamon *et al*., 2008), TIU of raw soybean (up to 84 TIU/mg) was higher than that of the legume seeds here studied (6.52-16.28 TIU/ mg). However, heat treatment of soybean can reduce TIU by 57-90% showing values sometimes below the level found in the SBM studied (0.61 TIU/ mg). Capetillo *et al*. (2001) found a significantly lower in vitro digestibility of raw soybean seeds compared with SBM, and Martínez *et al*. (1998) found a negative relationship between trypsin inhibitor activity (TIA) and in vitro protein digestibility in green beans (*Phaseolus vulgaris*). Although most compounds with protease inhibitor activity are heat-labile, it has been found that the thermostability of trypsin inhibitors in legume varies not only with the legume source but also with the conditions used during processing (pH, humidity, time, temperature, pressure) (Frias *et al.*, 2000). Several authors have reported that cooking and autoclaving are more effective to reduce trypsin inhibitors activity than dry heating (Khalil & Mansour, 1995). Guillamon *et al*. (2008) showed that trypsin inhibitor isoform varied with legume species and variety.

TIA up to 3.2 mg TI/g diet did not affect pancreatic secretion of nitrogen or protein or pancreatic chymotrypsin activity in young pigs (Gabert *et al*., 1996). Similarly, according to Batterham *et al*. (1993), growing pigs may tolerate dietary levels of at least 4.7 mg TI/g, without significant negative effects. On the other hand, a lower dietary maximum tolerance level for fattening pigs of approximately 0.5 mg TI/g was recommended by Huisman & Tolman (2001). Taking into account the average dietary inclusion level of the selected raw grain legumes here studied, it can be assumed that these threshold levels are unlikely to be exceeded in pig diets.

Lectins (haemagglutinins) are glycoproteins which are able to reversibly bind to specific sugars and glycoproteins on the surface of cells in the gut wall, thereby interfering with nutrient breakdown and absorption. This reaction is manifested, *in vitro*, by agglutination of red blood cells from various animal species and therefore, lectins have traditionally been measured by their haemagglutinating activity (Grant, 1991). Lectins are extremely specific; different types having different interactions on toxicity, blood groups, mitogenesis, digestion and agglutination (Pusztai *et al*., 2004; Muzquiz *et al*., 2012).

The SBM here used showed the higher lectin content but well below the positive control (*P. vulgaris* cv. Processor, 11,111 HU/g) which is in line with literature values (Grant, 1991; Trugo *et al.,* 1999). In this study, no haemagglutinating activity was found in samples from *C. arietinum* or *V. narbonensis* (Table 4). Therefore, all these legume crops can be considered as non-toxic in terms of lectin content.

Phenolic compounds and antioxidant activity

Plant phenolic compounds are diverse in structure but are characterised by hydroxylated aromatic rings. They are categorised as secondary metabolites, and their function in plants is often poorly understood. The total phenolic content usually varies with the type of legume and cultivar (Martín-Cabrejas *et al.,* 2009). The content determined in the samples here analysed (Table 4) is

similar to those reported for other pulses (Aguilera *et al.,* 2011).

The high antioxidant activity of the SBM sample can be attributed to their content of isoflavones and other phytochemicals with antioxidant activity or by the formation of Maillard reaction products during the previous heat treatment of the soybean (Chuckwumah *et al.,* 2013). Polyphenols contained in the diet of Iberian pigs (grass, acorns, etc.) have shown interesting properties for pig production since their antioxidant activity prevent fats oxidations contributing to conservation of dry-cured products (Rey *et al.,* 1997; López-Bote *et al.,* 2000). This is considered a key in meat quality, particularly in those Iberian pigs raised in extensive rearing systems in the meadows. In addition, polyphenols are important for improving the health status of the animals, since these possess anti-inflammatory and antimicrobial effects in the gut, and act as stimulants of the immune system (van Hees, 2012).

γ-glutamyl-S-ethenyl-cysteine (GEC)

Narbon vetch (*Vicia narbonensis*) is a legume very well suited for producing straw and grain for livestock due to its favourable agronomic characteristics. Narbon vetch constitutes a good supplement to animal diets because of its high protein content. Nevertheless, the grain has a poor palatability for animals and some deleterious effects on kidney and red blood cells have been described in pigs and broilers (Davies, 1987; Wali *et al.,* 2005) but no toxicity has been shown in sheep (Jacques *et al.,* 1994). Eason *et al.* (1990) observed that a 10% inclusion of narbon seed in the diet reduced the intake a 4% and the live a weight 2.6% as compared to soybean meals. Most of detrimental effects of narbon vetch on animals have been attributed to the presence in the seeds of the dipeptide GEC (Sanchez-Vioque *et al.,* 2011) an off-flavour precursor closely related to similar compounds in chives (*Allium schoenoprasum*) and onions (*Allium cepa*) (Enneking, 1995). GEC makes raw narbon beans unpalatable to animals and humans (Bryant & Hughes, 2011; Sanchez-Vioque *et al.,* 2011). This dipeptide has a role as a storage compound and a defence function against insects, predators, viruses, and even microorganisms. It could be possible to control GEC content in narbon beans through the selection of cultivars that do not accumulate sulphur in seeds, since GEC content was shown to be higher in seeds of plants grown on sulphur-rich soils and on alkalinic, saline or clayey soils (Berger *et al.,* 2003).

This compound is specific to this legume and does not appear in SBM and other studied legumes. Table 4 shows its content in the two cultivars of *V. narbonensis*. Arias *et al.* (2005) found that its content in the seeds ranges between 15 and 31 mg/g in 21 *V. narbonensis* cultivars collected in different Spanish and Italian regions. Castleman (2000) found GEC in a range of 4.1 to 37.7 mg/g in accordance with the results obtained in this work (table 4). As the *V. narbonensis* studied in this work (ICARDA-2470 and ZU-154) have an average GEC content lower than that reported in the literature to have negative effects in birds fertility and reproductive performance in pigs, these seeds might be included in monogastric diets as a potential substitute of soy up to the mentioned 10%.

L-canavanine

Canavanine (2-amino-4 guanidoxy butyric acid) is a non-protein amino acid with a similar structure to arginine and it is the main free amino acid in *V. ervilia* seeds, making it important in animal nutrition. Table 4 shows the canavanine concentration in the two samples of *V. ervilia* studied. Tschiersch & Hanelt (1967) reported percentages of L-canavanine in seed that ranges from 1.5 to 2.6 mg/g. Sanchez-Vioque *et al.* (2008) determined canavanine in flowered vetch (*Vicia articulata*) and they ranged from 2.7 to 6.7 mg/g. Berger *et al.* (2003) found in seeds of *V. ervilia* concentrations of canavanine varying from 0.4 to 1.1 mg/g that may constrain the end use of the grain, due to pigs are sensitive to concentrations as low as 0.08% in their diet. Its toxic effects in mammals are not fully known, although they seem to be fewer than expected, perhaps because of canavanine is degraded by certain bacteria in the digestive tract (Cacho *et al.,* 1989). As canavanine is high in nitrogen, its main function could be to store nitrogen for embryo growth. Taking into account the data obtained in this work and the mentioned sensitive limit to L-canavanine concentration in pig diets (0.08%), the inclusion limits in feed formulations for *V. ervilia* Taranto and *V. ervilia* Villanueva would be up to 40 and 15%, respectively.

Vicine and convicine

The pyrimidine glycosides, vicine and convicine, are compounds presents in the genus *Vicia*, and their aglycone derivatives, divicine and isouramil, are responsible for an haemolytic anaemia occurrence, in man namely favism. *V. narbonensis* cultivars were the only analyzed seeds that contain vicine (Table 4). Cardador-Martinez *et al.* (2013) found in ten different varieties of *Vicia faba* amounts ranging from 2.88 to 6.10 mg/g of vicine and from 0.60 to 1.68 mg/g of con-

vicine. Burbano *et al.* (1993) analyzed forty lines of *V. faba* that amounts range from 3.6 to 8.7 mg/g for vicine and between 0.09 and 0.72 mg/g for convicine. The amounts accumulating in *V. faba* seeds were much greater than in *V. narbonensis* seeds. Ramsay and Griffiths (1996) reported vicine and convicine concentrations in *V. faba* similar to those found in this work for *V. narbonensis* (0.3 mg/g for vicine and traces for convicine).

The low concentration of vicine and convicine in *V. narbonensis* is important from a nutritional point of view, since these compounds are hydrolysed by the intestinal microflora to divicine and isouramil. In birds, they result in a decrease in egg weight and size, weaker egg shells, an increased number of blood spots in the egg, and a decrease in fertility and hatchability of eggs. In pigs, they have been related to reduce reproductive performance. Vicine and convicine, and their degradation products appear to have no direct effect on nutrient digestion and metabolism. The levels of vicine and convicine vary considerably between varieties of faba beans; the most effective means to reduce the levels of these ANFs in faba beans is the selection of varieties with low contents (Dublecz, 2011). The amounts found in this work are very low in comparison to those mentioned as threshold levels for pigs and birds. Accordingly, the varieties of *V. narbonensis* and *V. ervilia* here studied might be included safely in feed formulations as potential soy substitutes.

β-N- oxalyl-L-α, β-diaminopropionic acid (β-ODAP)

Lathyrus species, particularly *L. sativus*, have been implicated in the paralysis of humans and animals (ruminants and monogastric) (Hugon *et al.,* 2000) known as "lathyrism" or "neurolathyrism". The causative agent for lathyrism is the non-protein amino acid β-ODAP, also referred to as β-N-oxalylamino-L-alanine (BOAA). This compound was analyzed in a previous work using a CZE method (Sacristán *et al.,* 2015). In *L. cicera* seeds, samples ZL41 and ZL02, the β-ODAP content (data not shown in table 4, in Sacristán *et al.*, 2015) was similar (0.79 and 0.80 mg/g, respectively) and below the recommended toxic values for nutrition (Anadon-Navarro *et al.,* 2010). Taking into account the average dietary inclusion level of *L. cicera* in feed formulations (usually as a partial substitute of soy) and the low ODAP levels here found, *L. cicera* seeds (ZL41 and ZL02) might be used safely in feed formulations as a potential substitute of soy.

As conclusions, the biochemical characterization of the minor crops here studied allow us to explore their limits of inclusion in feed formulations in connection with ANFs levels (i.e. *V. narbonensis* <10% in diets for monogastrics) in order to replace SBM or soy concentrates, the main protein sources in feed compounding. It is very important to highlight that feed formulations are complex cereal-legume based mixtures in which the limit of inclusion of any given feedstuff is highly conditioned by the required final values of protein or energy, the cereal-legume amino acid profile complementation and the use of additives such as pure amino acids, high valuable fats or enzymes such as phytases (taking into account that cereals also add phytates to formulations). As the legume crops here studied are known good sources of valuable protein and energy, they should be considered by the feed industry as potential candidates to replace partial or totally SBM or soy concentrates. Knowledge on their ANFs content, as carried out in this work, is a key factor to establish their practical utilization in feed formulation.

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