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ORIGINAL PAPER

The effect of year and variety on the nutritional value of *Camelina sativa* meal

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Abstract

Camelina [*Camelina sativa* (L.) Crantz] is emerging as a promising crop due to its high oil content of seeds with a predominance of unsaturated fatty acids, and good protein content of defatted meal. This study investigated the nutritional composition of defatted camelina meal obtained from four varieties (three from Canada and one from Austria) grown in the Padana Plain (northern Italy) for four consecutive years (2016–2019). Statistical analyses were performed based on the collected data. Principal Component Analysis (PCA) revealed distinct nutritional profiles among varieties and growing seasons. Calena, the Austrian variety, exhibited good nutritional quality and stability over the years. Pearl showed an intermediate year-to-year stability but promising values for trypsin inhibitors, condensed tannins and *in vitro* digestibility. The anomalous quantity of rainfall recorded during the early stage of seed development in 2019 allowed us to state that extreme climatic events can significantly affect the seed composition of the camelina varieties. This makes it clear that varietal and environmental factors need to be considered to produce a crop that can be fed to livestock.

Keywords Antinutritional compounds · Climatic conditions · Defatted meal composition · Variety

Introduction

Camelina [*Camelina sativa* (L.) Crantz], also named false flax or gold of pleasure, is an annual species belonging to *Brassicaceae* family. Seeds are used by humans since from Neolitic Age as testified by some archeo-botanical remains and the mummy of Silkeborg, Denmark [1–2]. Although camelina is a minor crop, it has received increasing attention from researchers in recent decades. This is due to the search for oilseed crops with low agronomical input, adapted to marginal areas, resistant to biotic and abiotic stresses and useful in industrial processes of economic importance [3–5]. In addition to good agronomical performances [6–7], camelina seeds are characterized by a very high oil content (up to 40% on dry matter basis) with a high percentage of polyunsaturated fatty acids (PUFA) [8]. The α-linolenic acid (ALA), an omega-3 fatty acid with beneficial properties on human health, accounts for more than 30% of PUFA [9–11]. Camelina oil was approved as a novel food by Health Canada in January 2010 (https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods/approved-products/camelina-oil-novel-food-information.html). The defatted meal, the by-product of

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oil extraction, has a high protein content, more than 30% on dry matter basis, with an amino acid composition comparable with that of soybean [12]. These properties make camelina seed suitable for food, feed, biofuel, and as a source of industrial precursors [13–15].

Reducing the demand for soybean in livestock production can make a significant contribution to the urgent need to find more environmentally friendly ways of producing feed, one of the aim of Farm to Fork strategy of EU 2020 (Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:52020DC0381 accessed 2 May 2024). The effects of a partial substitution of soybean, the main source of vegetal proteins in animal diets, with press cake obtained by several oilseed crops, such as rapeseed, canola, sunflower, etc., have been the object of several studies [16–18]. In this frame, camelina meal, obtained after oil extraction, has gained increasing attention being comparatively cheaper than soybean. In general, the percentage of defatted meals that can be safely added to feed is strictly related to the amount of undesirable compounds present in each species. The optimal threshold to be used is determined by taking into account animal health, feed palatability, which in turn affects animal acceptance, and the negative impact on the taste of final products such as milk, meat and eggs [19–22]. Like other members of *Brassicaceae* family, camelina seeds contain significant amounts of antinutritional compounds such as glucosinolates (GLSs), protease inhibitors, sinapine, phytates and condensed tannins [11, 16, 23]. These compounds limit the safe addition of defatted camelina meal in monogastric and ruminant diet. Previous studies showed the safety of adding low quantities of defatted meal in the diet of poultry, pigs, quails and cows [24–27]. However, the influence of genotype, annual climatic variations and abiotic stress on meal composition has been focused on changes in fatty acid profile and protein content [28–30]. Conversely, the information on antinutritional levels in relation to genotype and growing environment is still largely unexplored [11, 31]. The identification of the camelina varieties best performing over the growing seasons is a basic information for its wide use in feed production. This paper presents the results of biochemical analyses of defatted meal obtained, after solvent oil extraction, from four camelina varieties grown in the same location near Bologna (northern Italy) during the 2016 to 2019 growing seasons.

Materials and methods

The camelina varieties Calena (Austria), Pearl, Midas and Cypress (Canada), all spring-type, were analysed. They were grown for four successive years (2016–2019) at the experimental farm of University of Bologna located at Cadriano (44°33' N, 11°23' E) Bologna province, northern Italy. The experimental farm has a homogeneous and neutral soil type. Sowing was performed on the second decade of March in plots of 12 m^2. The layout of the field trials was a randomized complete block design with three replications. Seeds were harvested on the last week of June. Temperature and rainfall quantity were recorded at the growing site during the vegetative cycle of the plants. Seeds of the commercial varieties PR 45D01 and PR 46W14 of canola (*Brassica napus* L.) obtained from Pioneer (Italy), and seeds of NAV 555 and EM Brezza of soybean (*Glycine max* L.), kindly provided by Dr. M. Galbiati, were included in this study as references.

Biochemical analyses

5 g of seeds, randomly selected from each of the three field replicates of each variety and year, were mixed and successively ground using a commercial coffee grinder. The meal was defatted with hexane (1:10, w/v) at room temperature for two times, dried at room temperature and stored at − 20 °C until analysed. Seeds of the commercial varieties of soybean and canola were milled, defatted, and processed as described for camelina.

Crude proteins were extracted according to Li et al. [14] with some modifications. Defatted meal was mixed with NaOH 0.01 M (17 mg/ml) and extracted at room temperature for 2 h with continuous stirring. After centrifugation, aliquots of the supernatant were used for the protein quantification according to the Lowry method [32]. Phytic acid (PA) was determined following De Boland et al. [33] with minor modifications. Briefly, PA was extracted from the defatted meal with a solution 0.4 N HCl with 0.7 M Na_2SO_4 and precipitated by 15 mM $FeCl_3$ in 0.2 N HCl. The phosphorus content into the precipitate was spectrophotometrically determined, after acid digestion with sulphuric acid according to Chen et al. [34].

Glucosinolates (GLSs) were determined according to Pozzo et al. [11]. Briefly, GLSs were extracted from the defatted meal with ethanol 70% (v/v) and purified by loading on a DEAE Sephadex A-25 column (Sigma-Aldrich, Milan, Italy). Bound GLSs were desulfated by the addition of 10U of sulfatase (Type H1), eluted with 100% ethanol and dried at 50 °C. The pellets, resuspended in 20% ethanol, were analysed by HPLC as previously described [35]. Trypsin inhibitor (TI) content was determined according to Della Gatta et al. [36]. The TIs were extracted from defatted meals with a glycine buffer (pH 11) and quantified spectrophotometrically at 410 nm. The assays were carried out using bovine trypsin and benzol-DL-arginine-p-nitroanilide

hydrochloride (BAPA). The content was expressed as inhibitor units for dry defatted matter (TIU mg^{-1}).

Condensed tannins were extracted twice with 70% acetone (1:10, w/v ratio), evaporated to dryness and resuspended in 100% methanol. Condensed tannins were determined by the vanillin method using catechin as a standard [37].

Ash content was determined gravimetrically according to the AOAC method 923.03 [38]. Total phosphorus was determined colorimetrically [39]. Protein digestibility was assessed with the two-step *in vitro* protocol of Stodolak and Starzynska-Janiszewska [40]. Approximately 120 mg of defatted meal were incubated at 37 °C with freshly prepared solutions of pepsin and pancreatin, at acidic and basic pH, respectively. After dialysis against NaCl, the amount of proteins retained inside the dialysis tube was estimated by the Lowry method [32].

Protein Solubility Index (PSI %) was determined on defatted meals previously autoclaved at 121 °C for 20 min (1:5, w/v) and lyophilized. Proteins were extracted and quantified as described for the crude proteins. PSI was calculated according to the formula:

PSI\% = [(protein of defatted meal-protein of autoclaved meal)/protein of defatted meal] x 100.

Statistical analysis

ANOVA analysis was carried out to estimate the effect of the variety and the growing season on each parameter. The two-factor variance analysis was used to estimate the effect of the year x variety. Significant differences were determined at $p<0.05$. The experimental data were normalized before the Principal Component Analysis (PCA). All the analyses were carried out with the packet Statistica ver. 7.0 (StatSoft, Tulsa, OK, USA).

Results and discussion

In general, feedstuffs are produced by manufacturers mixing variable amounts of meal from different crops. Defatted meals, which are obtained by squeezing the seeds of oilseed crops, mainly soybean, are used to increase the protein content and hence the nutritional value of the feed. The use of defatted meals as source of proteins, should not disregard the amount of antinutritionals contained in the feed, as these compounds can appreciably reduce the nutritional value and negatively affect the animal health. Camelina is a promising source of proteins with a well-balanced amino acid composition [24]. However, its use in feed formulation is still limited. This may be partly due to a lack of knowledge about the levels of anti-nutritional compounds present in commercial varieties and how they may be affected by annual climatic variations in the growing location. To shed light on this issue the most important nutritional parameters of four varieties cultivated in the same location for four consecutive years were measured. As expected, a year-to-year variation was observed for all the parameters among and within the varieties (Table 1).

Overall, the highest variations were recorded for the parameters strictly related to the nutritional value, such as proteins, GLSs and *in vitro* digestibility. Although, all the varieties had protein contents higher than 50 g 100 g^{-1} (Table 1), Calena appeared to be the most promising, with the highest mean value and the lowest year-to-year variation (63.0 ± 1.08 g 100 g^{-1}). In addition, its average protein content was comparable to that of the two varieties of canola and NAV 555 soybean (Table 2).

Midas, with the lowest average value and the greatest variation among the growing seasons (49.0 ± 5.86 g 100 g^{-1}), had the poorest performance in terms of protein content. The protein values recorded in this study were significantly higher than the range (31.3–39.8%) reported in the literature for camelina cake [24, 41–42]. This significant disagreement can be attributed to the highly effective oil removal achieved by treating the meal with hexane, respect to the seed pressing. In fact, the cakes obtained by cold pressing still contain from 10 to 20% of oil [43–44].

Among the antinutritional compounds, GLSs, trypsin inhibitors (TIs), phytates and condensed tannins are present in camelina seeds at non-negligible levels. GLSs, when hydrolyzed, produce molecules (isothiocyanates, thiocyanates and nitriles) with detrimental effects on human and animal health [23]. TIs interfere with protein digestion inhibiting the protease enzymes [45], while phytates are negatively associated with the bioavalability of bivalent cations [46–47]. Contrasting roles are attributed to condensed tannins. They reduce the protein digestibility and the efficiency of converting nutrients into new body substances, but also have anticarcinogenic properties [48]. Previous studies reported a wide range of variation (13.2–42.3 µmol g^{-1}) for GLS suggesting the existence of a high genetic variation within the camelina germplasm for these compounds [49–50]. We found a narrow range of variation for GLS, in agreement with other authors who have analysed varieties not included in this study [11, 16, 31].

TIs are another important class of antinutritionals present in camelina meal as well as in other *Brassicaceae* species been related to environmental stresses, such as drought or salinity. The TI returned a narrower range of values (Table 1) as compared to those available in the literature 5.8–18.4 TIU mg^{-1} [50] or 11.8–26.6 TIU mg^{-1} [51]. Among the varieties studied, Pearl was the most interesting, showing the lowest TI value in every year (Table 1). As expected, the

Table 1 Results of defatted meals analyses. The values are expressed on dry matter basis

Table·1.·Results·of·defatted·meals·analyses.·The·values·are·expressed·on·dry·matter·basis.·¶

Variety	Year	Proteins·¶ (g·100g^{-1})	GLSs·¶ (μmol·g^{-1})	TIs·¶ (TIU·mg^{-1})	C.·tannins·¶ μgCE/mg	Phytates·¶ (μg·g^{-1})	P_{tot}·¶ (mg·g^{-1})	Phy/P_{tot}	Ash·¶ (g·100g^{-1})	PSI·¶ (%)	Dig·¶ (%)
Calena	2016	61.9±1.60	29.69±0.83	12.75±0.46	0.99±0.17	33.89±0.31	9.58±0.19	3.54	6.58±0.22	64.2±0.05	52.3±1.24
	2017	62.3±2.75	30.56±0.39	12.11±0.29	2.20±0.28	32.84±1.10	9.71±0.20	3.38	6.02±0.20	56.3±1.67	66.7±1.12
	2018	63.9±2.79	31.63±0.42	13.98±0.37	1.76±0.13	36.78±0.93	9.80±0.17	3.75	6.43±0.23	66.8±1.46	60.7±0.23
	2019	64.0±1.56	23.21±0.95	12.30±0.74	2.79±0.17	39.20±1.71	9.30±0.21	4.22	6.59±0.20	64.3±0.63	64.8±0.98
	mean	***63.0±1.08a***	***28.77±3.79a***	***12.8±0.84a***	***1.94±0.77a***	***35.68±2.88a***	***9.60±0.22a***	***3.72±0.36a***	***6.40±0.27a***	***62.9±4.58a***	***61.1±6.36a***
Cypress	2016	53.7±3.79	30.41±1.42	12.93±0.66	2.49±0.40	34.15±0.24	10.13±0.65	3.37	6.18±0.18	47.1±2.04	53.6±1.41
	2017	59.0±4.98	30.94±0.96	10.59±1.24	4.35±0.17	31.85±0.62	8.87±0.60	3.59	5.70±0.20	58.9±3.12	60.8±1.32
	2018	62.6±3.02	29.28±1.42	11.93±0.54	2.59±0.42	35.38±1.75	10.57±0.68	3.35	6.12±0.20	55.8±2.92	58.6±1.48
	2019	57.6±3.32	20.85±0.63	10.17±0.73	5.08±0.48	37.27±2.56	9.37±0.62	3.98	6.13±0.19	61.6±0.63	71.5±1.15
	mean	***58.2±3.69b***	***27.87±4.73a***	***11.4±1.26a***	***3.63±1.29ab***	***34.66±2.27a***	***9.74±0.76a***	***3.57±0.29a***	***6.03±0.22a***	***55.9±7.75a***	***61.1±7.53a***
Midas	2016	47.0±2.23	28.82±0.33	12.70±0.50	1.82±0.18	31.59±1.10	9.04±0.31	3.49	6.38±0.22	40.6±2.02	68.5±2.76
	2017	50.2±1.58	31.53±1.17	11.04±0.09	3.59±0.20	29.53±0.39	8.88±0.35	3.32	5.80±0.24	51.2±1.73	60.6±3.87
	2018	42.5±1.42	31.36±0.39	12.99±0.12	2.66±0.35	35.12±1.97	9.84±0.33	3.57	6.14±0.22	44.6±1.88	52.4±2.61
	2019	56.4±3.97	20.82±0.70	10.94±0.69	3.08±0.14	35.46±1.52	9.58±0.36	3.70	6.27±0.23	49.0±1.95	56.3±2.28
	mean	***49.0±5.86c***	***28.13±5.03a***	***11.92±1.08a***	***2.79±0.75a***	***32.92±2.86a***	***9.34±0.40a***	***3.52±0.16a***	***6.15±0.25a***	***46.4±4.72b***	***59.5±6.90a***
Pearl	2016	55.7±1.90	34.36±0.87	9.29±0.58	1.02±0.09	33.53±1.77	11.10±0.62	3.02	6.00±0.25	46.8±1.36	67.1±1.58
	2017	45.1±2.20	35.32±0.93	8.61±0.19	2.09±0.43	34.05±1.50	12.09±0.59	2.82	5.65±0.27	53.2±1.99	59.1±2.08
	2018	51.9±3.93	32.48±0.94	10.13±0.43	1.41±0.23	36.62±1.68	11.71±0.63	3.13	6.22±0.23	43.9±2.94	84.4±2.28
	2019	56.5±1.80	18.00±0.17	9.38±0.37	2.40±0.42	38.80±0.63	12.67±0.61	3.06	6.28±0.23	65.3±1.02	76.9±3.44
	mean	***52.3±5.20bc***	***30.04±8.11a***	***9.35±0.62b***	***1.73±0.63ac***	***35.75±2.44a***	***11.89±0.66b***	***3.01±0.13a***	***6.04±0.28a***	***52.3±9.52ab***	***71.9±11.10a***
	General·mean	*55.6±6.80*	*28.70±5.13*	*11.36±1.57*	*2.52±1.11*	*34.75±2.63*	*10.14±1.17*	*3.46±0.36*	*6.16±0.28*	*54.2±8.83*	*63.4±8.92*

GLSs·=·glucosinolates;·TIs·=·Trypsin·inhibitors;·C.·tannins·=·condensed·tannins;·P_{tot}·=·total·phosphorus;·PSI·=·Protein·Solubility·Index;·Dig·=·*in·vitro*·digestibility.·Values·followed·by·the·same·letter·within·a·column·are·not·significantly·different·(P>0.05)¶

Table 2 Results of meal traits recorded for the commercial varieties of canola and soybean. The values are expressed on dry matter basis

Variety	Proteins (g 100 g^{-1})	TIs (TIU mg^{-1})	Dig (%)
Canola			
PR45D01	66.20±2.70	5.73±0.91	78.46±3.45
PR46W14	65.31±3.06	3.68±0.86	74.80±1.52
Soybean			
NAV 555	66.68±2.93	40.55±2.28	70.32±2.20
EM Brezza	70.15±3.64	40.78±1.11	76.50±1.48

TIs Trypsin inhibitors, *Dig in vitro* digestibility

TI contents recorded in this study confirmed the significant differences for this antinutritionals among camelina, canola and soybean, with camelina values being twice as high as for canola, but a quarter as high as for soybean (Tables 1 and 2). This confirms the positive effect of a partial replacement of soybean with camelina on the nutritional value of feed especially for monogastric animals [44].

Phosphorus (P) is an essential mineral for plant. It is stored in seeds under different forms, phytate being one of them. No significant differences were recorded among the varieties for phytates, whereas Pearl had significantly higher values of P_{tot} (Table 1). Moreover, its lower phytates/P ratios suggested a not negligible effect of genotype on the targeting of P storage towards phytate synthesis. A high stability over the four years of trials together with not significant variations among the varieties were observed for condensed tannins and ash (Table 1). Compared to the values reported for whole seeds by Montero-Muñoz et al. [29] (3.69–4.93%) the ash values of the defatted meals resulted higher.

The meal nutritional value includes, in addition to protein content, other parameters such as the amounts of the antinutritional compounds, the profile and availability of amino acids and the protein functional properties (i.e.: digestibility, solubility, gelation, etc.). The *in vitro* digestibility is one of the laboratory methods that can give a good estimation of the nutritional value. The multi-enzyme assay returned a very wide range of values, from 52.34 to 84.45%, and a high variation for all varieties over the four growing seasons (Table 1). *In vitro* digestibility of Calena, Cypress and Midas was regularly lower than those of canola and soybean (Table 2), while Pearl exhibited remarkable values in 2018 and 2019 (84.4 and 76.9, respectively). The lack of a significant correlation between *in vitro* digestibility and TIs (Table 3) reduced the negative effect attributed to TIs, suggesting that the recorded digestibility for camelina could be the result of multiple factors. Further research is needed to clarify this issue as camelina meal contains a greater number of compounds than other crops and some of these may affect digestibility.

It is known, that a proper heat treatment of meal (boiled, autoclaved or oven toasted) in addition to protein denaturation has a positive effect on nutritional value because of the partial or complete destruction of some antinutritional compounds. The effect of autoclaving on camelina meal was

Table 3 Correlation coefficients among the meal traits analyzed

	Proteins	GLSs	TIs	C.Tannins	Phytates	P_{tot}	Ash	Dig	PSI
Proteins	1.00	− 0.33	0.23	0.03	0.34	− 0.23	0.42	0.07	**0.75***
GLS		1.00	0.08	− 0.43	**− 0.58***	0.03	− 0.44	− 0.23	− 0.41
TI			1.00	− 0.15	− 0.05	**− 0.61***	**0.58***	− 0.47	0.06
Tannins				1.00	− 0.01	− 0.44	− 0.37	− 0.04	0.20
Phytates					1.00	0.41	**0.55***	0.34	0.46
P_{tot}						1.00	− 0.13	0.45	− 0.02
Ash							1.00	0.04	0.26
Dig								1.00	− 0.01
PSI									1.00

*Significant at $p < 0.05$. *GLSs* glucosinolates, *TIs* Trypsin inhibitors, *C. tannins* condensed tannins

P_{tot} = total phosphorus; Dig = *in vitro* digestibility; PSI = Protein Solubility Index

Table 4 ANOVA results for the defatted meal traits. Mean square and significance are showed

Source of variation	DF	Proteins	GLSs	TIs	C. tannins	Phytates	P_{tot}	Ash	PSI	Dig
Year (Y)	3	0.0041	**0.0919***	0.0146	0.1040	**0.0169***	0.0018	**0.0055***	0.0088	0.0034
Variety (V)	3	**0.0379***	0.0030	**0.0434***	**0.1169***	0.0045	0.0347	0.0028	**0.0213***	0.0123
Y x V	6	**0.0205***	**0.0477***	**0.0290***	**0.1148***	**0.0107***	**0.0185***	**0.0042***	**0.0150***	**0.0077***

*Significant at 5%. *DF* degree of freedom, *GLSs* glucosinolates, *TIs* Trypsin inhibitors, *C. tannins* condensed tannins, P_{tot} total phosphorus, *PSI* Protein Solubility Index, *Dig in vitro* digestibility

estimated by determining the Protein Solubility Index (PSI) one of the available indicators reflecting the thermal denaturation of proteins. As shown in Table 3, PSI and proteins were found significantly correlated (0.75 $p < 0.05$), while no correlation was found with TIs as reported for soybean [52]. Experimental evidences showed that the autoclave treatment had a different effect on the four studied varieties (Table 1). In fact, the average PSI values of Midas was significantly different from the values of Calena and Cypress, while a wide variation over the years (43.9–65.3%) was recorded for Pearl. For soybean, PSI is a routinely used parameter to avoid under- or over-processing of meal used for feed. The experimental evidences collected in this study suggest the need for further studies to explain the different responses to heat treatment shown by the four varieties.

Effect of genotype and metereological conditions

The wide variation in the traits analysed in this study can be ascribed to the effect of genotype, environment or both. The ANOVA analyses evidenced that the genotype significantly affects proteins, TIs, condensed tannins and PSI, while the growing season had a significant effect on GLSs, phytates and ash (Table 4). On the other hand, all the examined traits were affected by genotype x year. Based on these statistical evidences, it can be inferred that both variety and growing location should be carefully selected in order to avoid undesirable effects on the nutritional quality of meal.

In order to better assess this issue, the experimental data were submitted to Principal Component Analysis (PCA). The first three components (PC1-PC3) accounted for 89.5% of the total variance. Condensed tannins followed by ash had the highest absolute loading on PC1 (2.2748 and 0.9605, respectively). GLSs and proteins mainly influenced PC2 (2.1630 and 0.9556 absolute values, respectively), while P_{tot} and digestibility were the main source of variation in PC3 (1.6281 and 1.4000, absolute values, respectively). The dispersion of the points in the 3D plot provided a visual representation of the differences among the varieties and, for each, how much the growing season affected the meal composition (Fig. 1).

The high stability of Calena in the northern Italian environment is confirmed by the overlap of the points representing the years 2016, 2017 and 2018 (c1, c2 and c3, respectively). The low dispersion of the points p1, p2 and p3, corresponding to Pearl's meal in the same three growing seasons, underlines the acceptable inter-annual stability as well as the nutritionally promising composition of its meal (highest GLS and P levels and lowest TI contents). Conversely, Cypress showed the highest inter-annual variability, as testified by the wide scatter of its points, and the complete isolation in the plot of the samples 2016 and 2018 (cy1 and cy3, respectively). The subgroup on the right side of the plot deserves special attention, as it groups all the 2019 harvest (Fig. 1, c4, m4, p4 and cy4).

A detailed analysis of Table 1 shows that for a large part of the parameters and regardless of the genotype, the values recorded in 2019 were appreciably different from those of the previous three years. For example, significant decreases in GLS contents, the highest phytate amounts (Table 1) and so on were observed in this year.

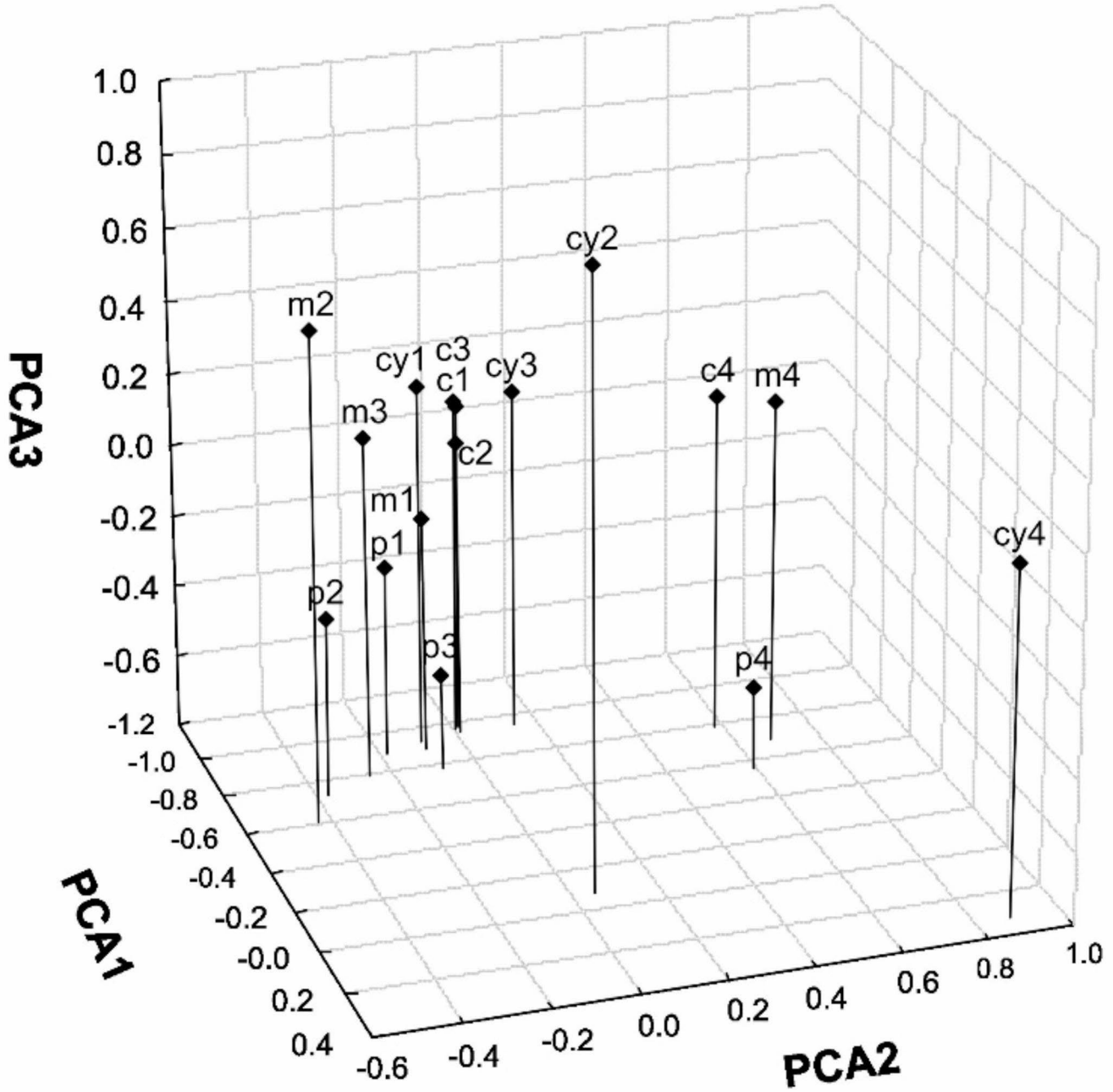

Fig. 1 3D scatterplot generated by PCA. Variety code: *c* Calena, *cy* Cypress, *m* Midas, *p* Pearl. Growing season code: 1-2016; 2-2017; 3-2018; 4-2019.

Based on these evidences, it can be speculated that the antinutritional compounds present in camelina seeds could be sensitive to climatic conditions during the vegetative cycle of the plant. Consultation of the annual regional report on climatic conditions (https://www.snpambiente.it/snpa/arpae-emilia-romagna/rapporto-idrometeoclima-emilia-romagna-dati-2019/ accessed 2 May 2024) shows that in May 2019 the climate in the area of the experimental field deviated appreciably from the average. In detail, the May 2019 was the wettest month of the trial, with two to three times the rainfall (Fig. 2) of the previous three years (2016–2018) and average temperatures below the 10-year average (Table 1S). May was crucial for camelina as it was the time plant flowering and seed development for the spring varieties, such as all those included in this study. Although the 2019 anomalous climate was accidentally included in our field trials, it allowed us to make an initial assessment of how extreme climatic events can affect the seed composition of camelina. For a large number of species belonging to

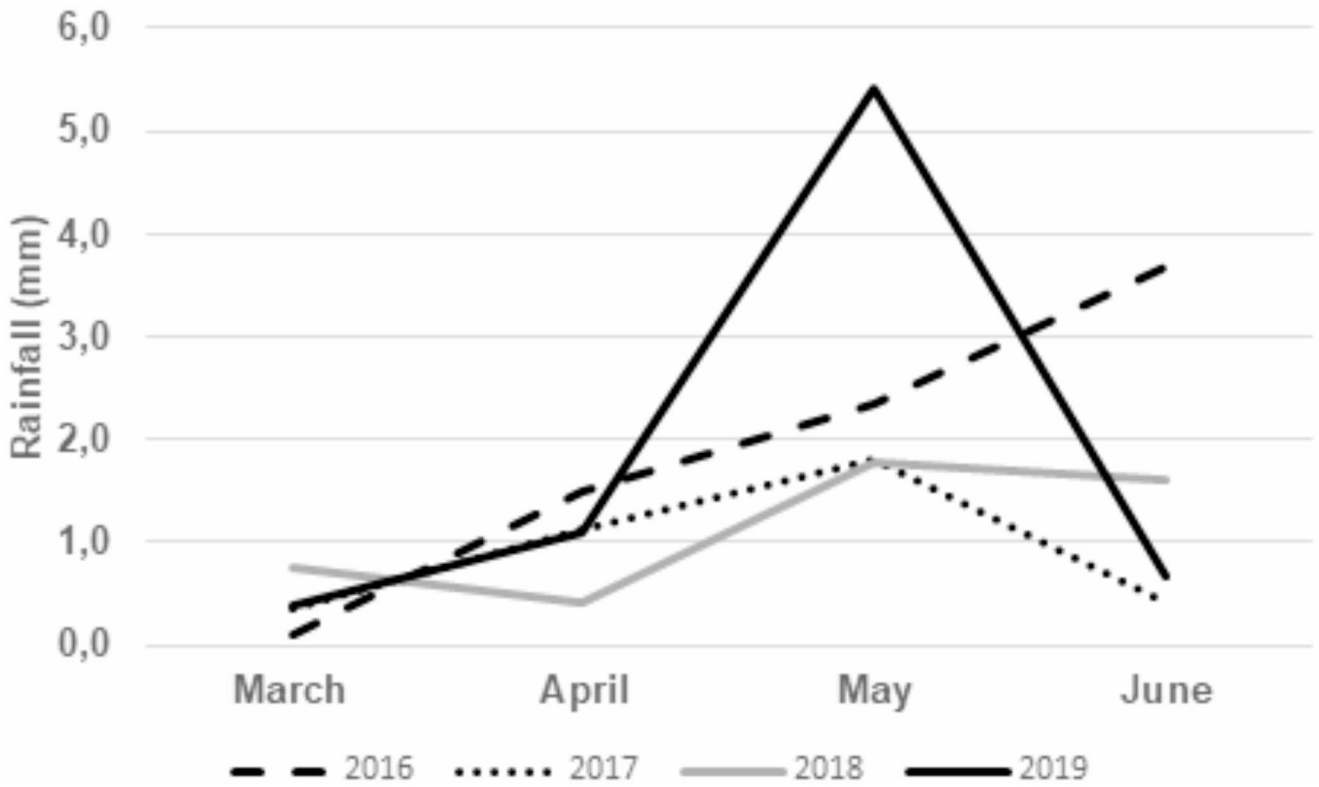

Fig. 2 Rainfall quantity during the cultural cycle in the years 2016–2019

different genera, significant effects of rainfall on the level of some antinutritional compounds have been described [53].

For *Brassicaceae*, the GLS content was shown to be genotype dependent, but also influenced by climatic factors. Charron et al. [54] reported that GLS concentration in ten varieties of *Brassica oleracea* is affected by cultivar, season and cultivar x season. Ciska et al. [55] recorded a significant increase in GLSs for three species of *Cruciferae* grown in water deficiency. The authors suggested a relationship between the GLS amount and the sulphur deficiency in the soil due to its leaching in the consequence of higher precipitations. The lack of sulphur, reducing the methionine synthesis a precursor of GLSs, might decrease the accumulation of the aliphatic GLSs such are those of camelina. The considerable reduction of the GLSs that we recorded in 2019 (Table 1) fits well with the explanation provided by Ciska et al. [55]. With regard to TIs, many authors observed, in several species and genera, an increase under conditions of drought stress [53]. The present study shows a similar response of camelina. As for GLSs, the higher rainfall quantity associated with May 2019 has led to a reduction of TI contents respect to the average of the three previous years (2016–2018). This was observed for Calena, Cypress and Midas which average values vs. 2019 are 12.95–12.30 TIU mg^{-1}; 11.82–10.37 TIU mg^{-1}; 12.24–10.94 TIU mg^{-1}, respectively. Conversely, Pearl resulted insensitive to the rainfall received during the early stages of seed development being the average value vs. 2019 substantially unaffected (9.34 vs. 9.38 TIU mg^{-1}).

Conclusions

The presented results suggest that the variety selection should be tailored to the environmental characteristics of the growing location to achieve optimization in meal nutritional quality. In the plain of north Italy, Calena showed the best stability in terms of meal composition. Among the Canadian varieties, Pearl showed a lower stability as compared to Calena but promising values for TIs, condensed tannins and *in vitro* digestibility.

Although, these results should be considered preliminary, due to the low number of varieties investigated the different response of genotypes to the same environmental pressure greatly suggests the necessity of further studies on the storage of antinutrional compounds in camelina seeds. With climate change bringing more extreme events, our results underline the necessity to increase the knowledge on the antinutrient storage within the camelina germplasm. This will be fundamental to set up the best strategy for genotypes with reduced amount of antinutritional compounds more adapted to a more sustainable livestock production.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest The authors declare no conflicts of interest.

Compliance with ethics requirements . This article does not contain any studies with human or animal subjects.

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