www.foodandfeed.fins.uns.ac.rs
FOOD AND FEED RESEARCH
Journal of the Institute of Food Technology – FINS
University of Novi Sad
ISSN:
2217-5369 (print),
2217-5660 (online)

UDK 636.085.2:[581.48+633.85:665.12

Original research paper

DOI: 10.5937/ffr49-41060

NUTRITIVE VALUE OF SERBIAN CAMELINA GENOTYPES AS AN ALTERNATIVE FEED INGREDIENT

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Abstract: Camelina has been used from ancient times, but recently has re-emerged as a valuable plant with the potential for successful replacement of conventional oilseed crops. The utilisation of camelina and its by-products in animal feed is a matter of scientific study due to their excellent nutritional potential. The present study aimed to investigate the nutritive value of two Serbian camelina seed genotypes (NS Zlatka and NS Slatka) as a potential alternative to commonly used oilseed crops in animal feeding. For that purpose proximate composition, fatty acid profile, amino acid profile and tocopherols were analysed. The study also included the investigation of the content of anti-nutritive compounds that can adversely affect the nutritional value of feed. The results showed that camelina seeds had a high amount of proteins (around 28%), amino acids and γ- tocopherols. Camelina genotypes were characterized by unique fatty acids composition, with its oil consisting of approximately 57% polyunsaturated fatty acids, of which the highest proportions were α-linolenic acid (~37%) and linoleic acid (~17%). An optimal ratio of n-6 and n-3 fatty acids (0.5) was also reported in this study. The concentration of anti-nutritional factors and heavy metals in camelina seeds was below the maximum set limit for feedstuff. To conclude, the investigated Serbian camelina genotypes can be used as a valuable source of proteins, essential fatty acids and tocopherols in animal nutrition and has a great potential to replace conventional oilseeds.

Key words: *oilseed, animal nutrition, protein source, ω-3 fatty acids, amino acids, tocopherols*

INTRODUCTION

With the growing human population, protein consumption has been considerably increasing, calling into question the sustainability of the food chain. The feed industry is under the great challenge of pleasing ever-growing demands for meat consumption. The constant need for more sustainable feed protein sources urges the scientific community to search for adequate alternatives to the most widely used soybean meal. In light of these efforts, camelina has

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been recognised as a promising candidate as a feed ingredient that could be successfully included in regular animal nutrition (Juodka et al., 2022; Riaz, Ahmed, Sizmaz & Ahsan, 2022).

Camelina (*Camelina sativa* (L.) Crantz) is a rediscovered protein and oilseed crop belonging to the *Brassicaceae* family, which was cultivated in the distant past (2000 BCE) in South-eastern Europe and Southwest Asia. It was grown sporadically in Europe as a conventional crop until the mid-20th century when it was replaced with more productive species like oilseed rape and sunflower (Zubr, 2010; Berti, Gesch, Eynck, Anderson & Cermak, 2016; Joudka et al., 2022). However, camelina has regained more attention in the last decade due to its numerous applications and agrotechnical and industrial benefits. Camelina may have many applications in biofuel production, materials, cosmetology, agrochemicals, food and feed industry, etc. (Mondor & Hernández-Álvarez, 2022).

The cultivation of this crop is suitable from an agricultural standpoint because of its short growing season and low fertilizer and water requirements. Also, camelina has broad environmental adaptability as it tolerates cold weather and drought and can be grown on marginal and saline soils compared to other oilseeds. Because of its phytochemical content, camelina is resistant to pests and some disease-causing agents, thus requiring lower amounts of pesticides and herbicides (Mondor & Hernández-Álvarez, 2022). Since climate change is one of the key threats to future agricultural production and food security, camelina could have an important role in the future of oilseed selection and breeding programs (Kuzmanović et al., 2021).

Camelina is cultivated due to its high content of protein, fat and valuable bioactive compounds, which deliver numerous beneficial effects on health. Its seed contains approximately 24-35% of crude proteins and 36% oil consisting of 40-60% polyunsaturated fatty acids, of which 35-40% is an α-linolenic fatty acid (Raczyk, Popis, Kruszewski, Ratusz & Rudzińska, 2016; Juodka et al., 2022). Camelina's nutritional profile is comparable to other widely cultivated oilseeds. Furthermore, camelina seed is a valuable source of natural antioxidants, especially tocopherols, phytosterols, phenolic acids, and flavonoids (Zanetti et al. 2021; Mondor & Hernández-Álvarez, 2022). Unusually high levels of n-3 fatty acids and their health benefits and relative stability make camelina oil suitable for human consumption as a part of functional foods (Berhow, Vaughn, Moser, Belenli & Polat, 2014). The extraction of oil from the seed with different solvents or by mechanical pressing results in a large number of by-products (cake and meal). These by-products are regarded as valuable sources of proteins and bioactive compounds in animal nutrition. Likewise, they are relatively cheaper feed ingredients in comparison to conventional products, especially soybean (Riaz et al. 2022). Recent studies have demonstrated that camelina by-products are an excellent source of proteins for fish and ruminants (Hixson et al., 2016; Halmemies-Beauchet-Filleau et al., 2017). Enrichment of animal diet with camelina seed and its by-products showed to be an effective way to modulate the fatty acid composition in milk, meat and eggs, thereby allowing the production of healthier products for consumers (Hurtaud & Peyraud, 2007; Ryhänen et al., 2007; Orczewska-Dudek & Pietras, 2019; Spasevski et al., 2020). Furthermore, numerous studies reported the improvement in blood biochemical profile with the dietary inclusion of camelina seed, cake and oil (Pietras & Orczewska-Dudek, 2013; Ciurescu, Ropota, Toncea & Habeanu, 2016; Anca, Hăbeanu, Lefter & Ropotă, 2019). However, the use of camelina seed and its by-products in animal nutrition is limited, especially in the diet for monogastric animals, due to the presence of glucosinolates, condensed tannins and phytic acid, which are ascribed as antinutritive factors.

Recently, two camelina genotypes were developed in Serbia from elite breeding material to expand the portfolio of species available in Balkan agriculture. These camelina genotypes have good production characteristics and are particularly developed to accommodate Balkan environmental conditions (Marjanović Jeromela et al., 2021; Čanak et al., 2022). On the other hand, little information is available on their nutritional and valorisation potential in animal nutrition. Therefore, the present study aimed to evaluate nutritive value including chemical composition, fatty acid and amino acid profile, as well as tocopherol content of two Serbian genotypes of camelina seed as an alternative feed ingredient.

MATERIALS AND METHODS

Materials

This research used two spring genotypes of camelina seed (*Camelina sativa*, L. Crantz), NS Zlatka and NS Slatka, developed at the Institute of Field and Vegetable Crops Novi Sad and registered by the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia in 2018. They present the first registered camelina genotypes in Serbia. Both genotypes are developed from elite breeding material by the process of self-fertilisation to conform to the Balkan environmental conditions. They are characterized by good production characteristics, with NS Zlatka demonstrating a higher oil yield than NS Slatka.

Chemical composition

Two samples of each camelina seed genotype were analyzed in duplicates for moisture (AOAC 934.01), crude ash (AOAC 942.05) and crude fibre content (AOAC 978.10) (AOAC, 1998). The crude protein content was performed according to AOAC 978.04 method, while crude lipid content was evaluated using AOAC 920.39 method. The content of macro and micro minerals was determined using SRPS EN ISO 6869:2008 method based on atomic absorption spectrometry. Determination of Pb, Cd As was performed by atomic absorption spectrometry (AAS) according to the accredited in-house method FINSLab-5.4-3M-004/13. An Advanced Mercury Analyser AMA 254 (Altec, Prague, Czech Republic) was used for Hg analysis based on the FINSLab-5.4-3M-005 method. The glucosinolate content of camelina seed was determined by the method MSZ-08-1908 (1989). Determination of condensed tannins was carried out as described: after defatting of 2 g of ground seed material with diethyl ether, the sample was extracted with 30 ml 70% acetone. The sample was subjected to ultrasound for 30 min at 45°C, then vortexed and centrifuged for 10 min at 10640 x g. The extraction procedure was repeated twice; then, the supernatant was collected, filtrated, evaporated to dryness and finally dissolved in methanol. Sample extract (1 ml) was mixed with 2.5 ml 1% vanillin in methanol and 2.5 ml 1% H_2SO_4. The reaction time was 20 min in the dark, and the absorbance was read at 500 nm. The results were expressed as mg catechin equivalent per g of sample. Phytic acid was determined using the phytic acid (total phosphorus) Megazyme assay (Megazyme, Wicklow, Ireland; catalogue number: K-PHYT 05/17), which is based on the measurement of phosphorus-released phytic acid, myo-inositol, and monophosphate esters by phytase and alkaline phosphatase. The results were expressed as mg of phytic acid per g of wet sample.

Fatty acid composition

Lipids from camelina seed were extracted with chloroform: methanol mixture (2:1) for 2.5h. Then, the lipids were converted to fatty acid methyl esters (FAME) with 14% boron trifluoride in methanol solution according to SRPS EN ISO 12966-2:2017 method. The profile of FAME was determined using Agilent 7890A gas chromatography (Agilent Technologies, Santa Clara, CA, USA) fitted with fused silica capillary column SP-2560 (100 m × 0.25 mm, d=0.20 μm; Supelco, Bellefonte, USA) and equipped with a flame ionization detection (FID). The injection volume was 1 μL and the split ratio was 1:30. Helium was used as a carrier gas. An initial column temperature of 140 °C was maintained for 3 min, followed by increasing it to 220 °C at a rate of 3 °C/min and holding it for 5 min. Finally, the column temperature was increased to 240 ºC at a rate of 2 ºC/min and held constant for 10 min. The detector and injector temperatures were set at 250 °C. The identification of FAME was done by comparing their retention times with those of authentic standards (Supelco 37 Component FAME Mix, Sigma-Aldrich, St. Louis, MI, USA). The content of individual FAME was expressed in per cent of the total identified FAME.

Amino acid profile

The amino acid composition was assayed by ion exchange chromatography using an automatic amino acid analyser Biochrom 30+ (Biochrom, Cambridge, UK). Firstly, the samples were hydrolysed with 6M HCl (Merck, Germany) for 24 h at 110 °C, and after cooling to room temperature, dissolved in 25 mL of Loading buffer (pH 2.2) (Biochrom, Cambridge, UK). Then, the samples were filtered through a filter of 0.22 μm and transferred into a vial to be analysed. The technique is based on amino acid separation using strong cation exchange chromatography, ninhydrin colour reaction and photometric detection at 570 nm. The exception was proline which was detected at 440 nm. The identification of amino acids

was done by comparison of retention times with those from Amino Acid Standard Solution (Sigma-Aldrich, St. Louis, USA). The results were expressed as a mass of individual amino acids (g) in 100 g of wet sample.

Tocopherols

Tocopherols were analysed according to standard SRPS EN 12822:2014 method HPLC-FLD (Agilent 1260 Infinity, Agilent Technologies, Santa Clara, CA, USA) equipped with normal phase column (Phenomenex Luna Silica, 5 μm, 250 mm x 4.6 mm), excitation at λex = 290 nm and emission at λem = 330 nm. The results were expressed as mg of tocopherols per 100 g of dried sample (mg/100 g).

Statistical analysis

Two camelina seed samples per genotype were analyzed in duplicates. To determine differences in camelina seed genotypes, a one-way analysis of variance (ANOVA) was carried out, followed by Tukey's post hoc test. Differences of $p < 0.05$ were accepted as representing statistically significant differences. Data obtained during the study were analyzed using Statistica Software, version 14 (TIBCO Software Inc, 14.0.0. 15, December 2020, USA).

RESULTS AND DISCUSSION

The results of the chemical composition of Serbian camelina genotypes are shown in Table 1. The presented results showed that analysed genotypes had similar chemical composition and were characterised by a high content of protein (~ 28%), lipid (~38%) and fibre (~ 18%). When comparing tested genotypes, it was observed that NS Slatka had slightly but significantly ($p<0.05$) higher protein and fibre content than NS Slatka. The crude ash content was approximately 3% for both genotypes. The results are in consistence with other literature data reported for Romanian, Canadian and Polish genotypes (Ciurescu et al., 2016; Krzyżaniak et al., 2019; Zając, Kiczorowska, Samolińska & Klebaniuk, 2020), while others observed higher protein and lipid content but slightly lower fibre content for Italian genotype (Peiretti & Meineri, 2007). Ciurescu et al. (2016) observed similar protein, lipid and ash content to those found in our study but lower fibre content (around 11%). Zhang et al. (2021) evaluated the quality of two camelina cultivars grown under different climatic conditions across different locations in China and reported protein and lipid content to be in the range of 21-36% and 26-35%, respectively. The differences in the chemical composition of seed can be ascribed to the variety and environmental factors (climatic conditions and soil) under which the crop was grown (Zubr, 2003a).

Table 1.
Chemical composition of two camelina seed genotypes (wet basis)

Parameters	NS Zlatka	NS Slatka
Moisture (%)	5.95 ± 0.10	5.84 ± 0.04
Crude protein (%)	27.07 ± 0.0[b]	27.95 ± 0.05[a]
Crude fat (%)	37.71 ± 0.14	38.25 ± 0.10
Crude ash (%)	3.52 ± 0.04	3.35 ± 0.02
Crude fibre (%)	17.47 ± 0.16[b]	18.46 ± 0.04[a]
Glucosinolates (μmol/g)	25.16 ± 0.0[a]	24.20 ± 0.04[b]
Condesed tannins (mg/g)	1.27 ± 0.35	1.54 ± 0.2
Phytic acid (mg/g)	3.12 ± 0.04	2.81 ± 0.12
P (%)	0.50 ± 0.02	0.49 ± 0.03
K (mg/kg)	11729.57 ± 11.36[b]	12029.32 ± 0.24[a]
Ca (mg/kg)	1735.06 ± 0.62[b]	1769.84 ± 0.73[a]
Mg (mg/kg)	2485.97 ± 0.14[b]	2806.85 ± 0.83[a]
Fe (mg/kg)	59.45 ± 0.12[b]	93.33 ± 0.10[a]
As (mg/kg)	<0.1	<0.1
Pb (mg/kg)	<0.025	<0.025
Cd (mg/kg)	0.0358	0.0463
Hg (mg/kg)	<0.1	<0.1

Different letters within row indicate significant different values ($p \leq 0.05$)

Camelina has a higher content of protein than that in sunflower, linseed, and rapeseed but lower than that in soybean; and has a lower level of lipid than that in linseed and sunflower but similar to that in rapeseed (Balalić et al., 2017; Zając et al., 2020; Riaz et al., 2022). The level of fibres is higher in camelina than that in linseed (7.9%) but significantly lower than that in sunflower (46.3%) (Zając et al., 2020).

Data on the content of antinutritive compounds is crucial for determining the inclusion limits of oilseed and its products in animal diets (Matthäus & Angelini, 2005). Glucosinolates are antinutritive secondary metabolites found in *the Brassicaceae* family. They are relatively non-toxic, and their degradation products can adversely affect the intake, growth, and reproductive performance of animals, as well as thyroid, liver and kidney functions (Matthäus & Angelini, 2005). The structure of glucosinolates from camelina seed is different from that in rapeseed. Glucosinolates from camelina are predominantly of glucocamelinin structure, consisting of 60-65% 10-methyl-sulfinyl-decyl glucosinolate, and 9-methyl-sulfinylnonyl glucosinolate and 11-methyl-sulfinyl-undecyl glucosinolate which are distributed in the amount of approximately 30 and 10%, respectively. It is assumed that the antinutritive effect of glucosinolates from camelina is lower than that from rapeseed products (Riaz et al., 2021). Also, there are some reports that glucosinolates from camelina may have some beneficial health effects, like protection from some types of cancer and immune-modulatory effect (Berhow et al., 2014). The concentration of glucosinolates in tested camelina seed genotypes was around 25 µmol/g which was lower than the maximum level (30 µmol/g) defined by Commission Regulation (EU) No. 1275/2013/EC (EU Commission, 2013) for camelina seed and its products in animal feed. The observed glucosinolate concentration was significantly lower in comparison to other oilseeds such as crambe or mustard (115 and 130 µmol/gm, respectively) (Matthäus & Angelini, 2005), while a similar amount was described in earlier investigations for rapeseed by-products (Kormanjoš, Popović, Kostadinović, Marjanović-Jeromela & Spasevski, 2016). Variable concentrations of glucosinolates (15.2-24.6 mmol/kg) in camelina flour were observed by Russo and Reggiani (2012) when testing twelve spring camelina genotypes different by origin (Germany, Poland, Italy, Austria, Russia and USA). According to Yuan et al. (2017), whole camelina seed had lower glucosinolate content (14.1 µg/mg) than its by-products, such as seed meal and defatted meal (24.3 and 31.8 µg/mg, respectively), but higher than that in crude oil (7.55 µg/mg). NS Zlatka had slightly but significantly ($p<0.05$) higher glucosinolates amount than NS Slatka. Glucosinolate concentration highly depends on the climatic conditions, variety, fertilization program, type of soil and sulphur content (Mathäus & Zubr, 2000; Berhow et al., 2013).

Condensed tannins are known for lowering the digestibility of feed in ruminants and non-ruminants. The mechanism behind this effect is the tannins reaction with proteins, enzymes or amino acids after enzymatic or non-enzymatic oxidation and the formation of different complexes (Matthäus & Zubr, 2000). No significant differences ($p>0.05$) were observed in the content of condensed tannins between tested camelina seed genotypes. The content of condensed tannins for both genotypes was relatively low (1.27-1.54 mg/g) and comparable to that found in other studies (Matthäus & Zubr, 2000; Berhow et al., 2014). Low concentrations of condensed tannins are also observed in screw-pressed camelina cake and camelina expeller (2 and 1.9 mg/g, respectively) (Kahindi, Woyengo, Thacker & Nyachoti, 2016; Kiarie et al., 2016). According to Russo and Reggiani (2012), the content of condensed tannins in camelina seeds differing in origin was in the range 1.92-4.39 mg/g. The low concentration of condensed tannins in the present study indicates that there should be no negative effects of the dietary inclusion of camelina seed on animals, as they show toxicity at the above 1% amount in animal diets (Russo & Reggiani, 2012). Even present in small amounts, tannins are observed as health-promoting compounds in animal nutrition as they have antimutagenic, anticarcinogenic and antimicrobial potential (Russo & Reggiani, 2012).

Phytic acid has antinutritive properties, and as it binds to metallic cations such as K, Mg, Ca, Mn, Fe and Zn forming insoluble complexes, which reduce the bioavailability of micronutrients from the diet (Juodka et al., 2022). On the other hand, it is believed that phytic acid shows hypocholesterolemic, anticarcinogenic and antioxidant effects (Ram, Narwal, Gupta,

Pandey & Singh, 2020; Juodka et al., 2022). The content of phytic acid in NS Zlatka and NS Slatka was 31.2 and 28.1 mg/g, respectively, which was similar to the previously reported results (Russo & Reggiani, 2012), while higher than that observed by Matthäus & Angelini (2005). The tested camelina genotypes showed similar contents of phytic acid to those in sunflower (Joudka et al., 2022), but higher than those in soybean and lupine seeds (14.3 mg/g and 13.8 mg/g, respectively) (Hídvégi & Lásztity, 2002). Generally, the concentration of phytic acid in camelina seed is lower compared to other members of the *Brassicaceae* family, predominantly used in animal nutrition (Riaz et al., 2022).

Among macro-minerals, camelina seeds had the highest content of potassium, while calcium, magnesium and phosphorus were less distributed. Regarding micro-minerals, camelina seeds contained iron in small amounts. When comparing the two tested genotypes, NS Slatka was characterised by a significantly higher ($p<0.05$) potassium, calcium and magnesium content than NS Zlatka. The mineral content of Serbian camelina genotypes was lower compared to the results of Zając et al. (2020) for camelina grown in Central Europe. According to Riaz et al. (2022), cold-pressed camelina meals can contain a higher concentration of macro-minerals compared to camelina seed or camelina cake.

Table 2.
Fatty acid profile of two camelina seed genotypes

Fatty acid (%)	**NS Zlatka**	**NS Slatka**
Myristic acid (C14:0)	0.1 ± 0.02	0.1 ± 0.01
Palmitic acid (C16:0)	5.8 ± 0.02	5.6 ± 0.04
Palmitoleic acid (C16:1)	0.1 ± 0.02	0.1 ± 0.01
Stearic acid (C18:0)	2.6 ± 0.0[a]	2.4 ± 0.0[b]
Oleic acid (C18:1n9c)	13.5 ± 0.01[b]	13.9 ± 0.06[a]
Linoleic acid (C18:2n6c)	16.7 ± 0.03	16.9 ± 0.03
Arachidic acid (C20:0)	1.5 ± 0.01	1.4 ± 0.01
Gondoic acid (C20:1n9)	16.0 ± 0.08	16.1 ± 0.01
α-linolenic acid (C18:3n3)	36.8 ± 0.05	37.0 ± 0.11
Eicosadienoic acid (C20:2n6)	2.1 ± 0.01	2.0± 0.01
Erucic acid (C22:1n9)	3.1 ± 0.01[a]	2.8 ± 0.01[b]
Eicosatrienoic acid (C20:3n3)	1.7 ± 0.01	1.7 ± 0.01

Different letters within row indicate significant different values ($p \leq 0.05$)

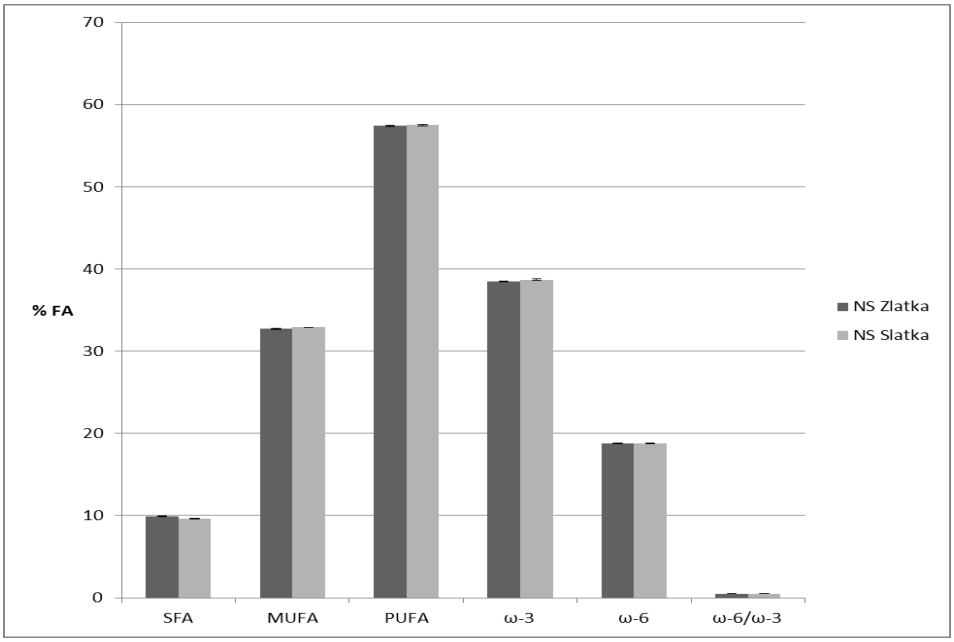

Figure 1. Major fatty acid groups in two camelina seed genotypes
(FA - fatty acids; SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids)

Some plants that belong to the *Brassicaceae* family possess the ability to accumulate heavy metals. Several studies investigated the potential for phytoremediation using plants from this family (Putnik-Delić, Maksimović, Zeremski & Marjanović-Jeromela, 2013). Accumulation of heavy metals in the seed is the result of unfavourable climatic conditions and environmental pollution (Matthäus & Zubr, 2000). Some heavy metals may even contaminate animal feed during feed processing (Dai et al., 2016). Even at low concentrations in feed, heavy metals can cause serious health problems in animals due to bio-accumulative potential. In this study, the concentrations of heavy metals were below the maximum allowed limit defined by Commission Regulation (EU) No. 1869/2019/EC (EU Commission, 2019). A higher cadmium concentration was observed in camelina grown in Scandinavia and Irish-Scottish region (Matthaüs & Zubr, 2000). Considering the very low concentration of heavy metals in analysed camelina seed genotypes, their utilisation in animal feed should not adversely affect animal health.

The fatty acid profile of camelina seed is presented in Table 2 and Figure 1. Fatty acid analysis showed that camelina seed exhibited a very beneficial fatty acid profile with the highest percentage of polyunsaturated fatty acids (PUFA) (~57%) followed by monounsaturated (MUFA) (~33%) and saturated fatty acids (SFA) (10%) (Fig. 1). Among SFA, the most dominant fatty acid was palmitic acid (C16:0), while stearic, arachidic, and myristic acid were less distributed. Gondoic and oleic acid were the major MUFA in camelina seed, accounting for approximately 16 and 14%, whereas erucic acid was present in a lower amount (~3%). Erucic acid is considered a limiting factor in determining the suitability of oilseeds application in animal feed. Even though the share of this acid is higher than that in canola (Joudka et al., 2022), it is below the maximum acceptable level in feedstuff established by Commission Regulation (EU) No. 1881/2006/EC (EU Commission, 2006). Analysed camelina seed genotypes had a similar fatty acid profile, while NS Slatka had slightly but significantly ($p<0.05$) lower levels of stearic acid, SFA, and gondoic acid, while higher content of oleic acid than NS Zlatka. Moreover, camelina seeds were abundant in n-3 and n-6 PUFA, of which the highest proportions were α-linolenic acid (~37%) and linoleic acid (~17%). These fatty acids are essential as they cannot be synthesized by the body thus must be included in the diet. From a nutritional viewpoint, α-linolenic and linoleic fatty acids are important as they have many beneficial health effects, thereby increasing the biological value of the feed. By incorporating n-3 PUFA in animal feeding, it is possible to produce animal products with an improved n-3 fatty acid profile. Omega-3 PUFA is essential for the proper functioning of the organism and is associated with improved growth performance, feed efficiency, reproductive performance, and immunity, as well as the reduced negative impact of heat stress and inflammation of vascular tissue (Alagawany et al., 2021). Camelina seed has favourable content of α-linolenic acid compared to many other oilseeds used as feed components. The share of α-linolenic acid in camelina seed is higher than that in soybean, sunflower, hempseed and rapeseed but lower compared to that in linseed (Čolović et al., 2015; Zając et al., 2020; Joudka et al., 2022). Based on the level of α-linolenic acid, camelina is considered the second highest-growing plant convenient for animal nutrition (Joudka et al., 2022). Comparable profiles of fatty acids were reported for camelina cultivated in Poland and China (Krzyżaniak et al., 2019; Zheng et al., 2021). Higher content of oleic acid but lower levels of α-linolenic acid and gondoic acid was reported for Iranian camelina genotypes (Piravi-Vanak et al., 2022). Furthermore, Günç Ergönül and Aksoylu Özbek (2018) found lower content of oleic acid but a higher share of linoleic acid in oil from camelina grown in Turkey. A lower content of α-linolenic acid but a higher level of erucic acid was reported for the Romanian genotype (Ciurescu et al., 2016). Compared to other oilseeds, camelina has a lower content of oleic and linoleic acid than sunflower seed, while similar contents of linoleic and lower oleic acid content than linseed (Zając et al., 2020). The amount of n-3 and n-6 fatty acids are not the only determining factor in the nutritive evaluation of oils from oilseeds but also their ratio because these groups of fatty acids compete for their biosynthetic enzymes during metabolic processes in the body. Therefore, the balanced ratio of n-6 and n-3 fatty acids is a vital factor for health management and prevention of certain diseases, referring to a higher proportion of essential n-3 PUFA (Simopoulos, 2016). In this study, the ratio of n-6 and n-

3 PUFA was 0.5, considered optimal according to Simopoulos (2002). The obtained results indicate that the analysed Serbian camelina genotypes are abundant sources of essential fatty acids, suitable for use in the diet for livestock and aquaculture. As shown in Table 3, the proteins in camelina seeds are rich in amino acids accounting for 80.47-81.60 g/100, of which essential amino acids (EAA) constitute 28.04-28.15 g/100g.

Animals must obtain EAA from diets as they cannot synthesize them in the body. EAA are necessary for normal body functioning, and their deficiency in diets can cause the development of certain diseases.

Therefore, their importance in animal nutrition should not be disregarded and great attention should be paid to the diet formulation to balance protein and amino acids from plant proteins to meet animal requirements (Bătrîna et al., 2020).

Protein feed ingredients, which are mostly plant-based, are relatively deficient in some essential amino acids, especially methionine, which is often the first limiting amino acid for protein synthesis. Camelina seed has an appreciable amount of EAA, which makes it a significant protein and EAA source for ruminantsand non-ruminants. In this paper, leucine was the most dominant EAA (6.75-6.78 g/100g), followed by lysine (6.14-6.15 g/100g) and valine (5.85-5.90 g/100g). The concentration of frequently deficient methionine was 1.44-1.48 mg/100g. NS Slatka was higher ($p<0.05$) in histidine but lower in phenylalanine level when compared with NS Zlatka. The concentration of non-essential amino acids (NAA) was 52.43-53.45 g/100g, which was predominantly glutamic acid (10.99-11.21g/100g), followed by arginine, aspartic acid and proline. Other identified NAA were abundant in the concentration of less than 6 g/100g. NS Zlatka had significantly ($p<0.05$) higher content of aspartic acid, alanine, glycine and total NAA than NS Slatka. The amino acid profile was comparable to that of Romanian camelina cultivars grown at eight different localities across Germany, England, Ireland, and Scandinavia (Zubr, 2003b; Bătrîna et al., 2020). The exceptions were threonine, phenilylalanine and glutamic acid which were less distributed in our study. Zubr (2003b) compared the amino acid profile of camelina seed with that in soybean, rapeseed and linseed, and observed that linseed had a higher content of cysteine, arginine, aspartic acid, glutamic acid, and glycine, but had lower content of lysine than camelina seed. Compared to camelina seed, soybean had a higher level of lysine, aspartic acid and glutamic acid, but a lower level of valine, whereas rapeseed was lower in arginine and aspartic acid, but higher in lysine and glutamic acid.

Table 3.
Amino acid profile of two camelina seed genotypes

Amino acids (g/100g protein)	**NS Zlatka**	**NS Slatka**
Leucine	6.78 ± 0.20	6.75 ± 0.07
Valine	5.95 ± 0.06	5.80 ± 0.06
Threonine	0.69 ± 0.05	0.79 ± 0.01
Isoleucine	3.79 ± 0.10	3.70 ± 0.04
Lysine	6.15 ± 0.07	6.14 ± 0.05
Methionine	1.48 ± 0.05	1.44 ± 0.01
Histidine	2.40 ± 0.02[b]	2.60 ± 0.03[a]
Phenylalanine	0.92 ± 0.00[a]	0.82 ± 0.01[b]
EAA	28.15 ± 0.03	28.04 ± 0.05
Glutamic acid	11.21 ± 0.15	10.99 ± 0.06
Aspartic acid	6.97 ± 0.00[a]	6.51 ± 0.06[b]
Proline	6.81 ± 0.04	6.91 ± 0.10
Alanine	4.22 ± 0.05[a]	4.09 ± 0.02[b]
Arginine	7.84 ± 0.06	7.86 ± 0.07
Glycine	5.59 ± 0.00[a]	5.50 ± 0.02[b]
Serine	3.73 ± 0.06	3.68 ± 0.04
Tyrosine	3.65 ± 0.17	3.43 ± 0.05
Cystine	3.44 ± 0.01	3.47 ± 0.09
NAA	53.45 ± 0.06[a]	52.43 ± 0.08[b]
TAA	81.60 ± 0.77	80.47 ± 0.28

Different letters within row indicate significant different values ($p \leq 0.05$); EAA - essential amino acids; NAA - nonessential amino acids; TAA - total amino acids

Table 4.
Tocopherols in two camelina seed genotypes

Tocopherol content (mg/100 g)	**NS Zlatka**	**NS Slatka**
α-tocopherol	1.41 ± 0.13	1.08 ± 0.03
β-tocopherol	<0.1	<0.1
γ-tocopherol	18.43 ± 0.89	19.82 ± 1.14

Tocopherols are natural antioxidants in vegetable oils, preventing lipid peroxidation by scavenging peroxide radicals. They protect PUFAs, which are considered unstable toward oxidative processes. Individual tocopherol isomers differ in their antioxidant activities and biological roles, whereby α- and γ-to-copherol have been reported to have the greatest antioxidant potential (Ratusz, Symoniuk, Wroniak & Rudzińska, 2018; Kiczorowska et al., 2019). Tocopherols occur in various amounts depending on the varietal properties, environmental conditions and processing conditions (Günç Ergönül & Aksoylu Özbek, 2018). According to Kirkhus et al. (2013), higher temperatures and lower precipitation during plant flowering induced higher levels of tocopherols, while N fertilization caused a decline in tocopherol content. Furthermore, different thermal treatments applied on oilseeds are reported to reduce the content of tocopherols (Kiczorowska et al., 2019).

The content of tocopherols in Serbian camelina genotypes is presented in Table 4. According to the results, there were no significant ($p>0.05$) differences in the content of tocopherols between the genotypes. The dominant tocopherol isomer observed in NS Zlatka and NS Slatka was γ-tocopherol (18.43 and 19.82 mg/100g, respectively), while α-tocopherol was less distributed in these genotypes (1.41 and 1.08 mg/100g, respectively). It was reported that γ-tocopherol was the major tocopherol isomer in camelina seed and linseed, while camelina seed had a higher concentration of γ-tocopherol as reported by Kiczorowska et al. (2019). Since both oilseed species have similar fatty acid profiles characterized by a high share of PUFA which are sensitive to lipid oxidation, higher γ-tocopherol in camelina seed could indicate more pronounced antioxidative potential and oxidative stability. It was observed that sunflower seed contains mainly α- and γ-tocopherols, while safflower contains a considerable amount of α-tocopherol (Günç Ergönül & Aksoylu Özbek, 2018; Kiczorowska et al., 2019). The content of α-tocopherol was similar to that observed in camelina grown in Slovenia, Norway and Iran, while a lower amount (0.2 mg/100g) was reported for the Polish genotype (Hrastar, Abramovič & Košir, 2012; Kirkhus et al., 2013; Kiczorowska et al., 2019; Piravi-Vanak et al., 2022). A variable amount of α-tocopherol (1.83-8.79 mg/100g) was reported by Günç Ergönül and Aksoylu Özbek (2018) for Turkish camelina cultivars. The content of γ-tocopherol in the present paper was significantly lower than the values observed for camelina cultivated in Northern Europe and Central Europe, with the Turkish genotype reaching the highest level of 136.4 mg/100g (Zubr & Matthaüs, 2002; Kirkhus et al., 2013; Günç Ergönül & Aksoylu Özbek, 2018; Kiczorowska et al., 2019). On the other hand, Toncea et al. (2013) Preported comparable results for the Romanian camelina genotype with ours.

CONCLUSIONS

In summary, tested Serbian camelina seed genotypes showed an excellent nutritional profile due to an appreciable amount of proteins, amino acids, and γ-tocopherols and moderate content of minerals. Likewise, camelina seeds were abundant with polyunsaturated fatty acids, linoleic acid and especially, with α-linolenic resulting in an optimal n-6/n-3 ratio. Additionally, the concentration of heavy metals in camelina seeds was very low to deliver any adverse effects on animal health. However, since this research did not investigate camelina seed genotypes over various growing regions and seasons, additional studies should be carried out to confirm the safety of this oilseed. Although the concentration of antinutritive compounds in camelina seeds was below the maximum allowed for feedstuff, their inclusion level in animal nutrition should be carefully monitored. Finally, Serbian camelina genotypes can be used as a source of proteins and bioactive compounds and a suitable alternative to commonly used oilseed crops in animal fee-

ding. Further research should be directed toward optimizing the inclusion level of camelina seed and its by-products in animal diets.

ACKNOWLEDGEMENTS

This research was financially supported by the Provincial Secretariat for Higher Education and Scientific Research, Autonomous Province of Vojvodina (Project no. 142-451-2609/2021-01/02), as well as by the Ministry of Education, Science and Technological Development, Republic of Serbia (451-03-68/2022-14/200222).

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NUTRITIVNA VREDNOST SRPSKIH GENOTIPOVA LANIKA KAO ALTERNATIVNIH SASTOJAKA HRANE ZA ŽIVOTINJE

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Sažetak: Lanik se gaji još od davnina, ali u poslednje vreme ponovo postaje predmet interesovanja zbog svog potencijala da zameni konvencionalne uljarice. Usled izvrsnog nutritivnog potencijala, iskorišćenje lanika i njegovih sporednih proizvoda prerade u hrani za životinje predstavlja predmet naučnih studija. Cilj ovog istraživanja bilo je ispitivanje nutritivne vrednosti semena dva srpska genotipa lanika (NS Zlatka i NS Slatka) kao potencijalne zamene uobičajeno korišćenih uljarica u ishrani životinja. U tu svrhu, rađene su analize hemijskog sastava, određivan je masnokiselinski i aminokiselinski profil kao i sadržaj tokoferola. Takođe, određivan je i sadržaj antinutritivnih materija koje mogu da umanje kvalitet hrane za životinje. Dobijeni rezultati su pokazali da seme lanika sadrži značajnu količinu proteina (oko 28%), aminokiselina i γ-tokoferola. Pokazano je da ispitivani genotipovi lanika poseduju karakterističan masnokiselinski sastav, pri čemu je ulje sadržalo oko 57% polinezasićenih masnih kiselina od kojih su najzastupljenije bile α-linolenska (~37%) i linolna (~17%) kiselina. Takođe je utvrđen i optimalni odnos n-6 i n-3 masnih kiselina (0.5). Sadržaj antinutritivnih materija i teških metala u semenu lanika bio je ispod maksimalne dozvoljene granice za hranu za životinje. Zaključak ovog istraživanja je da se ispitani srpski genotipovi lanika mogu koristiti kao vredan izvor proteina, esencijalnih masnih kiselina i tokoferola u ishrani životinja, kao i da poseduju potencijal da postanu zamena konvencionalnih uljarica.

Ključne reči: *uljarice, ishrana životinja, izvori proteina, ω-3 masne kiseline, aminokiseline, tokoferoli*

Received: *09 November 2022/* ***Received in revised form:*** *29 November 2022/* ***Accepted:*** *06 December 2022*

Available online: *December 2022*