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INDUSTRIAL HEMP (*Cannabis sativa* L.) FOR A SUSTAINABLE FEEDING OF RUMINANTS: NUTRITIONAL CHARACTERIZATION AND EFFECTS ON ANIMAL PERFORMANCE

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GLOSSARY

ADF = Acid Detergent Fiber

AIA = Acid Insoluble Ash

ALA = Alpha Linolenic Acid

CBD = Cannabidiol

CP = Crude Protein

CLA = Conjugated Linoleic Acid

DM = Dry Matter

EU = European Union

FA = Fatty Acid

GLA = Gamma-Linolenic Acid

HS =Hemp Seeds

HSC =Hemp Seed Cake

LA = Linoleic Acid

LCFA = Long-Chain Fatty Acids

MCFA = Medium-Chain Fatty Acids

MUFA = Mono Unsaturated Fatty Acids

NDF = Neutral Detergent Fiber

OA = Oxalic acid

PUFA = Polyunsaturated Fatty Acids

SCFA = Short-Chain Fatty Acids

SFA = Saturated Fatty Acids

THC = Tetrahidrocannabinol

UFA = Unsaturated Fatty Acids

WHC = Water Holding Capacity

ABSTRACT

Industrial hemp (*Cannabis sativa* L.) stands at the intersection of agriculture, legislation, and diverse applications, embodying a multifaceted crop with a rich history and a promising potential. Beginning with its legal framework, industrial hemp's status varies across countries, notably within and outside the European Union, reflecting the evolving perspectives on cannabis-related products. Exploring the plant's historical significance, taxonomy, current varieties, and cultivation practices provides a comprehensive foundation. Understanding the chemical composition of its different botanical fractions — stems, leaves, and seeds — unveils the versatility of hemp, showcasing its potential in various industries.

Delving into the by-products of hemp seeds, such as oil, cake, and meal, underscores the nutritional significance of this resilient plant. Hemp seed, in particular, emerges as a nutritional powerhouse, with its balanced profile of proteins, essential fatty acids, and other vital nutrients. Beyond human consumption, hemp demonstrates potential in the animal industry, with studies investigating its viability as feed for ruminants and other animals. The exploration of hemp's stem as a potential animal bedding material presents another utility, highlighting its sustainable and eco-friendly qualities in agricultural practices.

The aim of this work was to perform a complete overview of industrial hemp (*Cannabis sativa* L.) as a sustainable feed for ruminants. This included the chemical characterization of the whole plant, its botanical fractions and its derivatives (cake, meal, and oil). Also, the use of hempseed cake as feed for veals was tested. To finish with the use of hemp stems as an animal bedding material.

In essence, industrial hemp transcends its botanical origins, intertwining with legal considerations, agricultural practices, nutritional value, and innovative applications in the animal industry. This investigation concluded that industrial hemp is a versatile and nutritionally valuable resource for feeding ruminants and encourages further exploration for discovering its effect in animal health, behavior and performance.

INTRODUCTION

Industrial Hemp Legislation in the European Union and extra-EU

In the present day, the current industrial hemp law regarding its cultivation is regulated by each EU member state authority and the European Commission. In general, hemp is allowed to be grown in the EU.

The industrial hemp Legislation in the EU framework is under the common agricultural policy (CAP 2023-27) that supports farmers and ensures Europe's food security. The CAP includes provisions for the cultivation of hemp, including subsidies and support for hemp farmers. Also, some of the following regulations are involved in the productive chain of industrial hemp:

- Regulation (EU) N° 2021/215, which stablishes rules for direct payments and other support for farmers, including hemp farmers, under the common agricultural policy (CAP strategic plans)
- Regulation (EU) N° 1308/2013, which stablishes a common organization for agricultural market products, including hemp.
- Commission Delegated Regulation (EU) 639/2014, which stablishes the requirement to use certified seeds of the varieties listed in the "Common Catalogue of Varieties of Agricultural Plant Species"
- Commission Delegated Regulation (EU) 2016/1237 and EU Implementing Regulation 2016/1239, which establishes the import licenses rules for hemp.
- Commission Implementing Regulation (EU) 2016/1239, which lay down the rules for the application of the EU Regulation 1308/2013 of the European Parliament and of the Council with regard to the system of import and export licenses.
- Commission Regulation (EU) 2022/1393, which specifies the maximum levels of delta-9-tetrahydrocannabinol (THC) in hemp seeds and products derived therefrom.
- Commission Implementing Regulation (EU) 2022/1173, about the integrated administration and control system in the common agricultural policy.
- Council Directive 2002/53/EC, about the common catalogue of varieties of agricultural plant species.
- Council Directive 2002/57/EC, regarding the marketing of seed of oil and fibre plants.
- Council Decision 2003/17/EC, which describes the equivalence of field inspections carried out in third countries on seed-producing crops and on the equivalence of seed produced in third countries.
- Directive 2018/2001/EU, establishing a common system to promote energy from renewable sources like hemp could be.

- Regulation (EU) N° 2018/848, defining the EU rules on producing and labelling organic products like organic hemp.
- Regulation (EU) N° 2015/2283, the updated version of the Novel Food Regulation that came into force on 1st January 2018 where defines a novel food as any food that was not used for human consumption to a significant degree within the Union before 15 May 1997.

Regarding the industrial hemp legislation outside the EU, countries such as the US, Canada, Australia and China can be mentioned. In the US, hemp cultivation is under the legislations of 2014 and 2018 Farm Bills. The 2014 Farm Bill defined industrial hemp as plants with a THC threshold of 0.3% or less on a dry weight basis and allowed its production under specific conditions. The 2018 Farm Bill legalized its production an agricultural commodity and removed it from the list of controlled substances. These two simultaneously active Farm Bills creates an unlevel playing field in which sampling, testing procedures, harvest windows and testing frequency vary by state. In addition, on January 19, 2021 the US Department of Agriculture (USDA) published a final rule about the Domestic Hemp Production Program that provides regulations for the production of hemp in the US and it has been effective since March 22,2021 (Burton et al. 2022; Falkner et al. 2022). In Canada, cultivation of industrial hemp is currently regulated by Health Canada under the Cannabis Act. Health Canada licenses the cultivation of hemp under the Industrial Hemp Regulations. The Canadian Food Inspection Agency's (CFIA) regulates the import, export, certification and grading of industrial hemp seed under the following acts and regulations: seeds act (R.S.C.,1985, c.S-8), seeds regulations (C.R.C.,c.1400), Plant Protection Act (S.C.1990,c.22) and Plant Protection Regulations (SOR/95/212). For Australia, hemp laws vary by state. In China, only two provinces, Yunnan and Heilongjiang, have legislations allowing industrial hemp cultivation (Liu et al. 2023).

In South America, Colombia has recently changed its legislation, establishing mechanisms and procedures for the industrial use of hemp in pharmaceuticals, food, beverages, and dietary supplements. The most recent law in this country is the resolution 227 of 2022, from which Decree 811 of 2021 is given free rein and describes the regulation of its industrial use (Montero et al. 2023).

History, taxonomy, varieties, and cultivation of industrial hemp

Cannabis sativa is native from Asia and it is considered one of the most ancient cultivated plants probably first cultivated in China on 2700 BC (Farinon et al. 2020; Burton et al. 2022). It was introduced to Europe during the Bronze age (22th – 16th century BC). In the US, hemp was grown as an agricultural commodity from the early 1900s to the late 1950s and the USDA supported its production. Between 1914 and 1933, 33 states passed laws restricting legal production to medicinal and industrial purposes only. In 1937, the Marihuana Tax Act did not consider the difference between low THC plants (hemp) or high THC (marijuana) and defined hemp as a narcotic drug. This is why after 1943 the production of hemp started to decline and in the late 1950s no production was recorded for the US. In 1970, The Controlled Substances Act (CSA) defined *C. sativa* L. as marijuana and put it as a controlled substance under the Drug Enforcement Administration (DEA). In Europe, Italy was the second largest hemp producer in the world, after Russia, by having 100 000 hectares of hemp cultivation and was known as having the best quality hemp products. In 1938, Canada with the Canadian Opium and Narcotics Act prohibited the cultivation of hemp due to the presence of THC. In 1961, the United Nation (UN) endorsed and adopted the single convention on narcotic drugs, which established a universal system for limiting the cultivation, production, distribution, trade, possession, and use of narcotic substances to medical and scientific purposes, with a special focus on plant-derived substances, among which was cannabis. Later, in 1971, the UN established an international control system for psychotropic substances, among which is THC. Meanwhile, in line with these directives, in 1975 the Italian Republic issued the law n. 685/1975, introducing cannabis (intended as a drug product obtained from *C. sativa* L. plants) in the schedule of controlled substances (Farinon et al. 2020).

Since the last two decades, there has been a reintroduction of the *C. sativa* L. cultivation exclusively for industrial purposes, and in this context, Canada has been the first western country to restore this crop, followed by Europe and the US. Canada restored industrial hemp cultivation in 1994 when the Office of Controlled Substances of Health Canada began to issue licenses to hemp as a research crop and then, in 1998, the cultivation of hemp varieties containing less than 0.3% THC of the dry weight of leaves and flowering parts was legalized. In the EU, in 2013 the EU regulation n. 1307/2013 allowed the growth of *C. sativa* L. with low levels of THC for industrial purposes only. In the US, the 2014 Farm Bill allowed the USDA and certain research institutions to grow hemp under an agricultural pilot program. Then, the 2018 Farm Bill removed hemp and their products from its drug definition and the DEA schedule of controlled substances, opening the hemp industry for business (Farinon et al. 2020). Australia legalized hemp foods in November 2017 after a ban that had been in place since 1937 (Burton et al. 2022). Nowadays, hemp is cultivated in at least 47 countries for commercial or research purposes (Burton et al. 2022).

In Latin America, Cannabis plants arrived in the colonial era (16th century), when the kings of Spain promoted the development of the crop of hemp in their colonies. Between the end of the 19th century and the first decades of the 20th, Cannabis was also used as part of the medicines offered by pharmacies and drugstores in countries such as Mexico, Argentina, and Brazil. In the second half of the 19th century, some pharmacology investigations identified its psychoactive use and Cannabis began to be perceived as a vice and linked to criminal behavior. For such reasons, since the 20s, criminal legislation established prison for those who trafficked in countries such as Argentina (1919), Colombia (1939), Mexico (1920), Costa Rica (1928) and Brazil (1930) (Montero et al. 2023).

The taxonomy of C. sativa has been always a topic of discussion among researchers due to its high market value and regulatory factors among different countries (USDA-ARS 2020; Belwal & Belwal 2022) but mostly it is defined as shown below.

- Kingdom : Plantae (plants)
- Subkingdom : Tracheobionta
- Superdivision : Spermatophyta
- Division : Magnoliophyta
- Class : Equisetopsida
- Subclass : Magnoliidae
- Order : Rosales
- Family : Cannabaceae
- Genus : Cannabis
- Species : Cannabis sativa L.

Different varieties of hemp have been evolved as the result of plant breeding and selection programs. There are multiple types of hemp cultivars in commercial use, including open-pollinated populations, clones, and hybrids. Up to now, approximately 700 different varieties/cultivars of *Cannabis* have been identified and distinguished but only some of them are registered in the EU Plant Variety Database (Table 1). To be considered as industrial hemp, they all need to contain a THC level less than 0.2% of the reproductive part of the female plant at flowering according to the EU Regulation (EU 2013) and their cultivation purpose is basically to obtain fibre, seeds, and their derivatives. All cultivars bred for grain and fiber production are maintained as open-pollinated populations. There is a high genetic variability of *C. sativa* L but the varieties that genotypically and phenotypically differ are all interfertile so they can reproduce with one another (Farinon et al. 2020; Smart et al. 2021; Belwal & Belwal 2022).

Hemp is an annual herb growing during the warm season (Raman 1998). Is a fast-growing crop with the capability of growing in pesticide and herbicide-free environments, noticeable resistance to rodents, fungus and many types of weeds. The plant is heliotropic and flowering is critically controlled by the length of the photoperiod (Booth 2003).

The type of soil suitable for hemp cultivation is sandy loam, followed by clay loam soil. On the contrary, heavy clay soil and sandy soil are not well suited for this crop. The optimal soil pH for hemp cultivation is 6.0-7.5 and the mean temperature between 16-27°C. Preferably, the optimal soil for hemp should have good drainage and adequate water holding capacity, good aeration and residual nutrients (Sunoj et al. 2023).

Table 1. Industrial hemp cultivars registered and listed in the European Union Plant Variety Database and their origin

Country	Denomination	Country	Denomination	Country	Denomination
BG	AMX	FR	Santhica 23	LV	Pūriņi
BG	Arizona Dream	FR	Santhica 27	LV	Rodnik
BG	Auto Power 1	FR	Santhica 70	NL	Chamaeleon
BG	Midwest	HU	Balaton	NL	Enectaliana
BG	Morning Glory	HU	Cannakomp	NL	Ivory
BG	Northwest	HU	Fibrol	NL	MGC 1013
BG	OGK	HU	KC Bonusz	NL	Marcello
BG	Pain killer	HU	KC Dora	NL	Markant
BG	Strawberry H	HU	KC Virtus	NL	Uso-31
BG	Strawberry K	HU	KC Zuzana	PL	Beniko
BG	Troyanka I	HU	KCA Borana	PL	Białobrzeskie
BG	Western Cherry	HU	Kompolti	PL	Glyana
CZ	Felice	HU	Kompolti hibrid TC	PL	Henola
EE	Estica	HU	Lipko	PL	Matrix
ES	Delta-405	HU	Monoica	PL	Mietko
ES	Delta-llosa	HU	Tiborszallasi	PL	Rajan
FI	Finola	HU	Tisza	PL	Sofia
FI	Finola2	HU	Uniko B	PL	Tygra
FR	Dioica 88	IT	Asso	PL	Wielkopolskie
FR	Djumbo 20	IT	CS	PL	Wojko
FR	Earlina 8 FC	IT	Carma	RO	Armanca
FR	Epsilon 68	IT	Carmagnola	RO	Dacia Secuieni
FR	Fedora 17	IT	Carmaleonte	RO	Lovrin 110
FR	Felina 32	IT	Codimono	RO	Mara 21
FR	Felina 34	IT	Eletta Campana	RO	Olivia
FR	Ferimon	IT	Fibranova	RO	Ratza
FR	Fibrimon 56	IT	Fibrante	RO	Secuieni Jubileu
FR	Fibror 79	IT	Glecia	RO	Silvana
FR	Futura 75	IT	Gliana	RO	Succesiv
FR	Futura 83	IT	Villanova	RO	Teodora
FR	Mona 16	LT	Alive SK	RO	Zenit
FR	Muka 76	LT	Austa SK	SI	Fiona
FR	Nashinoïde 15	LV	Adzelvieši	SI	Fukal
FR	Nordria 3	LV	CFX-2	SI	Helena
FR	Orion 33	LV	CRS-1	SI	Marina
FR	Ostara 9	LV	Loja	SI	Stara Prekmurska

BG: Belgium, CZ: Czech Republic, DE: Germany, EE: Estonia, ES: Spain, FI: Finland, FR: France, HU: Hungary, IT: Italy, LT: Lithuania, LV: Latvia, NL: Netherlands, PL: Poland, RO: Romania, SI: Slovenia. Updated in October 2023.

A ploughing of 30-40 cm depth is recommended, while the seedbed preparation should be performed right before the sowing. Seeding dates are mostly determined by climatic factors such as soil temperature, moisture accessibility, as well as the photoperiod, which determines the duration of the vegetative process. The ideal sowing time to produce fiber is late March-early April. On the contrary, a dual-purpose crop needs could be postponed. The experiences conducted so far in Italy have observed that sowings done in mid-late May generally produce more seed than sowings done in mid-late May of April for both monoecious and dioecious varieties. Plant spacing in hemp is determined by the purpose, such as fiber or seeds. Hemp grown primarily for fiber is planted closely together to promote stalk elongation, greater yield and stronger fibers while reducing branching and flowering. Hemp is frequently planted using seed drills with row spacing ranging from 7.6 to 20 cm. The spacing between hemp plants grown for fiber ranges from 20 to 40 cm. Research has shown that 120 plants per square meter with an interrow spacing of 0.5 m produced high yields of the stem, seed, and inflorescence combined. The quantity of seed for sowing is higher for fiber than seed production. Seeding rate recommendations vary from 40 to 65 kg/ha for fiber hemp to reach 200–300 plants/m^2 and 20 kg/ha for seed. In general, sowing densities of about 30–40 kg/ha (100–150 plants m^2) are advised. Also, the ideal sowing depth vary from 1.9 to 3.2 cm depending on the soil type, soil preparation, available water, and seeding date. The goal is to prevent seeds from drying out before germination, so if sowing is done in a relatively humid environment, the sowing depth can rise up to 1-2 cm (Ahmed et al. 2022; Belwal & Belwal 2022; Blandinieres & Amaducci 2022; Viscovic et al. 2023).

Hemp germinates after 3 to 5 days of sowing. It has the ability to germinate at temperatures as low as 1-2°C but it is recommended that soil temperature reaches 10-12°C in order to ensure hemp's quick development that enhances its capacity to surpass weeds (Viscovic et al. 2023). After germination, hemp requires large amounts of nitrogen input for a good plant establishment. The recommended nitrogen level for growing dual-purpose hemp (seeds and fiber) is fertilization with 50-60 kg/ha. Higher levels of fertilization results in greater vegetative growth, higher height, scalar maturation and better crop establishment. It is advisable to divide in half the nitrogen fertilization, one at pre- seeding and the other post emergency (CREA 2019).

Hemp requires high soil water during the initial stage of root establishment. After that, a well-developed root system may allow hemp to withstand moderately drier conditions. However, the amount of water required for hemp cultivation depends on the agro-climatic region, genotype, soil characteristics, weather conditions, and evapotranspiration. Hemp is susceptible to waterlogging. Several studies have been conducted to understand the water requirements of hemp in different agroclimatic zones. For instance, studies conducted in Europe revealed that hemp needs 500-700 mm of water for growth and development. Meanwhile, in the vegetative stage, a minimum of 250–300 mm of water is needed for optimum growth. Cosentino et al. reported that 250 mm of water was required for monoecious early fiber genotypes and 450 mm for dioecious late genotypes grown in a semi-arid Mediterranean environment (southern Italy) (Cosentino et al. 2013). Another study conducted in southern Italy over two years with diverse genotypes showed that the replenishment of 66% of the water lost through evapotranspiration is required for excellent hemp production (Di Bari et al. 2024). Furthermore, the water requirement of hemp (435 mm) is higher than soybean and sunflower, but lower than sorghum (Sunoj et al. 2023).

In the Nordic climate, hemp blooms approximately 80-100 days after the sowing and the incipient ripeness of the seed occur after 120-140 days. Then, the harvest depends on the purpose use of the plant. For seed purposes, the harvest is carried out when the seeds are between 50% and 70% ripe, i.e. 2 to 4 weeks after the appearance of the first browned seeds surrounded by partially necrotized bracts. In this case, the timing of harvest has an impact on seed quality, particularly with regard to the lipid profiles. Typically harvest yields for hemp seed are: 750 kg/ha in Canada, 1000 kg/ha in Finland and 1000-1500 kg/ha in Sweden (Eriksson 2020). For fiber purposes, the stem yield and fiber quality will be significantly reduced with time. Harvesting for fiber occurs after flowering but before seed set and can be carried out with modified machinery. When harvested in the fall, August – October, the fibre quality will be high and it is worthwhile to separate the fibres from the core. The total stem losses are estimated at about 10% when harvested in the early fall. If hemp is harvested later in the fall or early winter, November-December, the stem would be more suitable for short fibre applications. The loss of stem material is estimated at 20% compared to the original stem biomass available. The harvest can also be performed in late winter and spring, January – May with the purpose to produce primarily energy. However, the stem losses are considerable and amount to approximately 40%. Hemp fibres from an early harvest are preferably used for textiles whereas hemp fibres from a late harvest may be good enough to be used as insulation materials. Hemp grown for dual purpose have more complex harvesting considerations, the seed heads are harvested first and then the remaining stalks are collected for getting the fibre. Thus, the harvesting equipment for hemp uses combine harvesters. These machines cut down the hemp plant at the base, cut off the flowering head for flower or seed processing, and sort the stalk for fiber processing (Burton et al. 2022; Ahmed et al. 2022; Belwal & Belwal 2022).

Chemical composition of hemp botanical fractions

As any other plant, hemp is composed by seeds, leaves and stems (Figure 1). The seed is an achene fruit, i.e. it contains a single seed with a hard shell, tightly covered by the thin wall of the ovary. It is light brown to dark grey, in some cases mottled, has an ellipsoid shape slightly compressed (2-6 mm x 2-4 mm) and it is smooth at touch. The stem of the hemp plant is grooved or furrowed to varying degrees, and hollow; when grown at a high plant density the stems are almost unbranched. At maturity the stem is 1 to 5 m high, depending on the cultivar and growing conditions. The leaves are big, serrated and composed of three to nine leaflets, have a dark green color and a rough surface. Seedlings have two sessile seed leaves; all subsequent leaves have a petiole. Both leaves of the first pair of true leaves have a single narrowly elliptic blade with serrate margins. A leaf from the second pair of true leaves has three serrate leaflets radiating from the tip of its petiole; a leaf from the third pair of leaves has five leaflets and so on, up to 9 to 13 leaflets (Booth 2003).

Seeds are the most nutritionally fraction of hemp as it offers a well-balanced profile of essential nutrients. They are an exceptionally rich high-quality protein, providing all nine essential amino acids required by the human body. Additionally, hemp seeds are abundant in healthy fats, particularly omega-3 and omega-6 fatty acids. Whole hemp seeds are also source of dietary fiber and boast an impressive array of vitamins and minerals, including vitamin E, magnesium, phosphorus, potassium, and zinc. Furthermore, hemp seeds are recognized for their content of bioactive compounds like phytosterols and antioxidants, which may have potential health benefits.

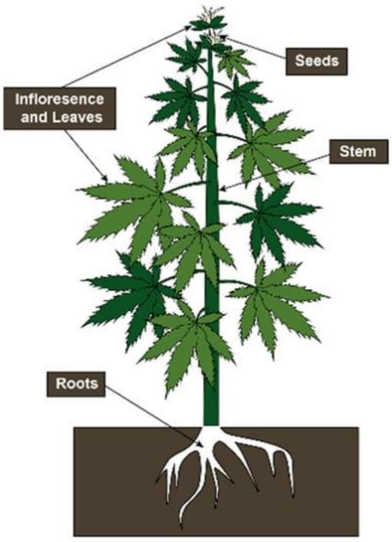

Figure 1. Hemp botanical fractions

Hemp leaves boast a complex chemical composition, featuring a variety of compounds that contribute to the plant's overall profile. While the concentration of cannabinoids like THC are low for industrial hemp, hemp leaves are still rich in other valuable cannabinoids, most notably cannabidiol (CBD). Additionally, hemp leaves contain a spectrum of terpenes, aromatic compounds and flavonoids that confers antioxidant properties. These leaves are an excellent source of protein, containing all essential amino acids. Hemp leaves are also rich in fiber. Furthermore, hemp leaves are a good source of vitamins, including vitamin A, vitamin C, and several B vitamins, contributing to overall well-being and immune system function. The leaves also contain essential minerals such as iron, magnesium, and zinc. Additionally, hemp leaves are known for their high content of omega-3 and omega-6 fatty acids in an optimal ratio.

The stem of the hemp plant is a rich source of fibers, like cellulose, hemicellulose, and lignin, which collectively contribute to the plant's robust and fibrous structure. While hemp stems may not provide a concentrated source of macronutrients like proteins or fats, it contains various phytochemicals, including cannabinoids, terpenes, and flavonoids.

(a) (b) (c)

Figure 2. Hemp stem (a), seeds (b) and leaf (c)

Table 2. Chemical composition of hemp botanical fractions

	Whole plant	Whole seeds	Leaves	Stems
Dry matter	70.3	94.1	88.9	95.0 ±1.8
CP	6.9	24.0	13.0	5.9 ± 3.0
Fat	2.7	30.4	8.9	4.0 ± 3.6
Ash	8.8	4.8	21.2	7.4 ± 1.5
NDF	81.6		44.7	79.1 ± 14.3
ADF	60.8		20.8	63.5 ± 16.0

Kleinhenz et al. 2020; Wang et al. 2022

Hemp seed: products of oil extractions (oil, cake, meal)

Hempseeds have a round shape, dark red‐brown color, with a variable diameter of 3 to 5 mm. Each seed is covered by a thin two-layered pericarp (the outer tube celled layer and the inner spongy parenchyma celled layer), an endosperm, and two cotyledons in its interior. Hempseed contains approximately 25% to 30% oil, 25% to 30% protein, 30% to 40% fiber, and 6% to 7% moisture. This proportion, however, varies largely among different hemp cultivars (Leonard et al. 2019).

Hemp seeds had gained great of interest because of its high nutritional value and the functional features of its bioactive compounds. They can be consumed raw or pressed into hemp seed oil, which has an excellent and unique fatty acid profile. Both seeds and oil are used for human food and animal feed. When pressing the oil out of the hemp seed there is a by-product produced called hempseed cake which is considered to be nutritionally equal to other protein sources (Eriksson 2007; Gibb et al. 2005; Zhao et al. 2022).

Figure 3. Hempseed cake

Conventional processing techniques are primarily aimed at extracting oil efficiently and to obtain an oil of good quality. The various methods for extraction of oil from oilseeds that have been applied to hemp seed include, solvent extraction, use of supercritical CO_2, microwave or ultrasound assisted processing and mechanical pressing. The most traditional processes for the extraction of hemp seed oil are solvent extractions and cold-pressing. Extraction of oil from hemp seed using solvent extraction, supercritical CO_2 or cold pressing resulted in oils with similar fatty acid composition. Pressing is one of the most simple, common and oldest method for obtaining hemp oil since it is considered natural and safe to use in food. Cold-pressing passes the raw seed material through a conventional screw press, without the addition of harsh chemical solvents or high heat treatments. At cold pressing, there is an amount of approximately 20% oil that is extracted whereby the remaining product is called hemp cake. After the first extraction, approximately 35% oil is left behind in the cake which is also rich in fiber and proteins. Superior quality oil is obtained from the first extraction process. Cold-pressed seed oil is free from chemical contamination and contains more beneficial components like natural antioxidants that prevent aging-associated diseases like heart diseases, cancer and other health problems (Ahmed et al. 2022). Due to high levels of polyunsaturated fatty acids, hemp seed oil is very susceptible to oxidation with virgin oil being especially sensitive. This is due to high contents of chlorophyll that acts as a photosensitizer (Burton et al. 2022). The oil extraction yield is associated with the extraction method. Cold-pressing extraction provides extraction yields of 27%−31.5% (Montero et al. 2023). One notable disadvantage of cold-pressed oil is the low yield potential of 60–80% of extractable oil (Vasantha et al. 2020).

Hemp seed oil may be extracted from whole or dehulled hemp seed. Approximately 30% to 35% of hempseed is composed of oil. The oil may then be further purified and refined. Cold-pressed oils from seeds have become more commercially because this process retains more of the beneficial components of the seeds, including valuable PUFA and bioactive substances, while minimizing degradative changes in the oil. Hemp seed oil is a good source of two essential fatty acids: linoleic acid (LA, 18:2 omega-6) and alpha-linolenic acid (ALA, 18:3 omega-3). The omega-6 and omega-3 exist in the ratio of 3:1, comprising the most desirable oil content beneficial for human nutrition (Ahmed et al. 2022). Unrefined hempseed oil is dark green in color, due to its chlorophyll content. (Leonard et al. 2019). Hempseeds oil has been available at specialty food stores across Europe and North America in the last decade. With its pleasant, nutty taste and slightly bitter aftertaste, hempseed oil is the most commonly used edible hemp derivative in cooking, even as an alternative to olive oil. Currently, hempseed oil is primarily advertised as a healthy product for salad dressings, or as a dietary supplement directly being taken (Xu et al. 2022).

Oil was traditionally considered the more valuable component of oilseed processing and the pressed cake and meal were considered to be by-products, initially used in the animal feed industry. There appears to be some confusion around the labelling of hemp seed cake and hemp seed meal in the literature and market as sometimes these terms are used interchangeably. However, hemp seed cake should refer to product obtained after mechanical pressing of oil while hemp seed meal is obtained after solvent extraction of the pressed cake. After expelling the oil using a screw press, the oil content in the cake obtained has 8.4-15.5% oil while the oil content in the de-lipidated hemp meal after solvent extraction is expected to be much lower (~1% oil) (Burton et al. 2022).

Table 3. Chemical composition of hempseed by-products obtained by cold mechanical (cake) or chemical (meal) extraction

(%DM)	Cake	Meal
Moisture		5.6
Fat	11.4 ± 1.1	11.1
CP	32.6 ± 2.3	33.5
Carbohydrates		42.6
NDF	44.9 ± 6.4	
ADF	35.9 ± 3.7	
Ash	7.2 ± 0.7	7.2

Klir et al. 2019; Mierlita 2018; Karlsson et al. 2010; Serrapica et al. 2019

Industrial hemp as feed for ruminants and other species

The interest in the use of hemp and its by-products in the animal industry is triggered by its high nutritional value of components. Hemp oil can be used as a supplement in feed mixtures for animals as a rich source of essential fatty acids (Cozma et al. 2015), while seeds (HS) and hempseed cake (HSC) can be used as a fat and protein source in animals´ diets (Mierlita 2019). Hemp seeds and cake can be used in all animal species and the whole hemp plant (including stalk and leaves) may be suitable for ruminants (EFSA 2011). The maximum incorporation rates in the complete feed could be 3 % in poultry for fattening, 5–7 % in laying poultry and 2–5 % in pigs for hemp seed and hemp seed cake, 5 % in ruminants for hemp seed cake and 5 % in fish for hemp seed (EFSA 2011). The whole hemp plant (including stalk and leaves) would be, due to its high fibre content, a suitable feed material for ruminants (and horses), and daily amounts of 0.5 to 1.5 kg whole hemp plant dry matter (DM) could likely be incorporated in the daily ration of dairy cows.

Hessle et al. (2008), fed young calves with diets based on mixed rations ad libitum and restricted supplement of protein feed made of HSC in experimental group and 50% of soybean meal and 50% of rolled barley in control group. Daily intake of NDF and fat were higher for calves fed HSC than for those animals fed soybean meal, with lower intake of starch. There weren’t any differences in liveweight gain of young calves. The NDF intake was higher by 31% when calves were fed with HSC compared to soybean as related to higher fibre content in HSC, which was also accompanied by fewer long particles in faeces. Hessle et al. (2008) suggested HSC as alternative protein feed for intensively fed growing cattle. Also, in the same research no differences were determined in carcass traits when steers were fed diets containing HSC compared to soybean meal.

Karlsson et al. (2010), fed dairy lactating cows with silage (494 g/kg DM) and concentrate mixtures (506 g/kg DM) formulated to contain increasing proportions of HSC: 0, 143, 233 and 318 g/kg DM. Milk yield was the highest when the cows were fed with addition of 143 g/kg DM compared to controls and cows fed with higher levels of HSC. Concentrations of urea increased with every additional level of HSC, while efficiency of converting dietary crude protein into milk protein decreased. Thus, inclusion of 233 or 318 g/kg DM of HSC had no benefits in milk performance.

Mierlita (2016) studied dietary supplementation with HS in the amount of 250 g/kg of concentrate mixtures when feeding mid-lactating ewes. This feeding increased the fat content in milk and energy corrected milk yield. Mierlita (2018) fed lactating ewes (DM intake 2.12 kg/d) with feed mixture containing 180 g of HS /d (as-fed) or with addition of 480 g of HSC /day (as-fed) All diets were isoenergetic. Beside milk fat, milk yield increased as well, when ewes were fed whether with HS or HSC compared to controls. Hemp seeds in diets improved fatty acid profile of ewes´ milk, especially with increased proportion of rumenic acid (conjugated linoleic acid-CLA, C18:2 c9 t11), and total n-3 fatty acids without detrimental effects on milk production in the study by Mierlita (2016). Hemp seeds increased concentration of ALA in ewes´ milk by 66% and hempseed cake increased ALA by 49% in the study by Mierlita (2018). Total saturated fatty acids (SFA), short-chain fatty acids (SCFA) and medium-chain fatty acids (MCFA) decreased, while PUFA, monounsaturated fatty acids (MUFA), and long-chain fatty acids (LCFA) increased.

Cremonesi et al. (2018), included 9.3% (on DM basis) of HS in diets for dairy goats with the aim to analyse the microbiome diversity of rumen liquor. HS inclusion promoted changes in rumen biohydrogenation pathway in dairy goats, such as an increase of C18:2 n-6 biohydrogenation intermediates, like C18:1 *t*6-8, C18:1 *t*9, C18:1 *t*10; C18:1 *t*12, compared to rumen liquor of goats fed linseeds which promoted more production of C18:3 isomers. Thus, the change of ALA and LA ratios in the diets affected the biohydrogenation pathway as reported by Shingfield (2010) as well. Hessle et al. (2008) studied the use of protein supplement in steers of 0.2-1.4 kg as fed of HSC or 0.7 kg rolled barley and 0.7 kg of soybean meal. Results showed that total lipid fatty acid profile differed in Longissimus dorsi muscle as affected by HSC supplement, which increased vaccenic acid (VA), oleic acid (OA, C18:1 n-9) and CLA concentrations, with decreasing n-6/n-3 ratio. Since PUFA proportions were not influenced by HSC, authors concluded that both diets are highly subjected to biohydrogenation in the rumen.

Addo et al. (2023) used six nonlactating Holstein cows fed with three isonitrogenous and isoenergetic diets: a basal partial mixed ration (PMR) with the addition of 10.2% DM hemp meal (HM), a basal PMR diet with the addition of 13.5% DM canola meal (CM), or a basal PMR diet with the addition of 6.16% DM canola meal and 6.25% hemp meal (HC). The replacement of canola meal with hemp meal in diets formulated from barley silage, wheat straw, and grass hay, and a CP content averaging between 10.2% and 13.5%, did not alter rumination or blood parameters and total-tract digestibility of DM and CP. A higher total-tract NDF digestibility in the CM diet was apparent compared with the HM diet. This may be due to the relatively low digestible NDF and high insoluble dietary fiber (29% DM to 32% DM) contents of the hemp seed meal protein used in the study. Cannabinoid contents in RF, blood plasma, urine, and kidney, liver, adipose, and muscle tissues of the cows were below detection limits. This shows that feeding hemp meal to nonlactating dairy cows does not lead to accumulation of cannabinoids in body tissues and biological fluids. A dietary inclusion rate of 10.2% of DM, hemp seed meal is a good and safe alternative for canola meal as a protein supplement for nonlactating dairy cows.

Kalaitsidis et al. (2023) evaluated the effects of diet inclusion with HSC on the performance of 20 lactating Holstein dairy cows during the first 40 days of lactation. They were divided in two groups with the same feed allowance of total mixed ration (TMR). The experimental group received a diet formulated with 3.5 % hemp cake, at a quantity of 1kg of hemp cake per cow per day. The inclusion of HSC did not affect milk production and composition. However, diet inclusion with HSC favorably influenced milk fatty acid profile by increasing total PUFA and preserving milk oxidation status indices.

Some studies on hemp products have also been done in monogastric species such as lying hens, broilers, ducks and cockerels. Mostly, they had focus in the possible transfer of unsaturated fatty acids to enrich the final product like meat and eggs. The inclusion of hemp oil up to 8% in layer diets and 6% in broiler diets did not negatively affect overall performance of birds and resulted in the enrichment of n-3 PUFAs and GLA in eggs and meat (Jing et al. 2017). A duck diet with hempseed cake produced exceptional-quality meat with an enriched content of n-6 GLA (Juodka et al. 2018). Incorporation of hemp seed into the diet increased cockerel's tibia strength (Skrivan et al. 2020).

Other potential uses of hemp plant

Another innovative approach of industrial hemp that is gaining prominence in the animal industry is the utilization of the stem or stalk as an animal bedding material. Hemp stalk consists mainly of cellulose and lignin and is rich in silica, a chemical that in nature is found in sand or flints. It is composed of two types of fiber: bast and hurds (Figure 4). The bast fibers are 5–40 mm long, represent 30-40% of the stalk and have been used to make paper for almost 2 millennia. The woody core fibers or hurds, representing 60-70% of a hemp stalk's total volume, are about 0.55 mm long so they are not considered for paper applications in which a length of 3 mm is ideal. These two types of fiber detach during a retting or a decortication process and they differ in its fiber composition (Table 4). In general, for each kilogram of hemp fibre produced, the industry gets 1.7 kg of hemp shivs as a by-product (Small and Marcus 2002; Ely et al. 2022).

Figure 4. Hemp stem fibres

Table 4. Bast and hurd hemp fiber composition

	Bast fibers	Hurd fibers
Cellulose (%)	73 - 77	34 – 48
Hemicellulose (%)	7 – 9	21 – 25
Lignin (%)	2 – 6	17 – 19

USDA 2019

The woody core (hurds, sometimes called shives) of hemp makes remarkably good animal bedding (Figure 5). In fact, hemp is considered a high-performance bedding material mostly used for horses, chickens, and pets. Between years 2010 and 2013, animal bedding represented 63% of the total hemp shiv applications with 45% of the market share for horse bedding and 18% for the other animals (Figure 6). It is considered an excellent litter for cats and other pets like hamsters, guinea pigs, rodents, bunnies and snakes this is why hemp hurds are sometimes molded into small pellets to get a better final product. In livestock shelters, not only hemp hurds but hemp straw is used as an option to barley or wheat straw because of its goo physical, chemical and biological properties (Table 5). (Airaksinen 2006; Russo et al. 2008; Bambi et al. 2018; Ely et al. 2022; Visković et al. 2023).

Figure 5. Animal bedding made from hemp hurds (Small & Marcus 2002)

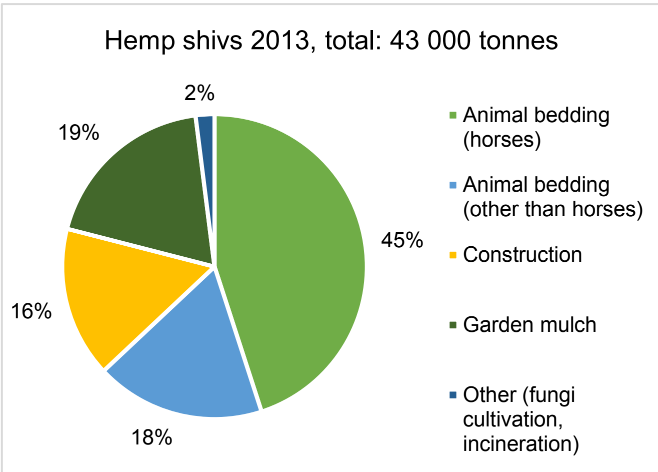

Figure 6. Applications for European Hemp Shivs from harvest 2013 (Carus & Sarmento 2016)

Table 5. Characteristics of bedding materials (score ranges from – to ++, where ++ is very positive, -- is very negative, and +- is neutral) for use in Freestall and compost bedded pack barns. *Can be used successfully if processed to < 25 mm

Material	Physical Properties	Chemical Properties	Biological properties	Suitability	
				Free Stall	Compost Bedded Pack
Hemp straw	+/-	+/-	++	X	X*
Barley straw	+	+/-	+-	X	X*
Wood chips	-	+	+	X	X
Wood shavings	++	+	+	X	X
Wheat straw	+	-	+	X	X

Ferraz et al. 2020

Hemp hurds and shives are highly absorbing because of its hydrophilic characteristics and great water absorption capacity (Table 6). They have been reported to be more absorbent than traditional wood or straw bedding options as they can absorb up to four to five times their weight in moisture (typically 50% higher than wood shavings). Unfortunately, the capacity to absorb water of hemp is reported under different names such as water holding capacity (WHC), water binding capacity or moisture binding capacity followed by different methodologies and making difficult to contrast them. If one calculates moisture binding capacity in terms of litter volume then the moisture uptake of fine wood shavings is around 10 times, with wood granulate and hemp shavings around seven times, higher than cereal straw. There are differences between the capacity to absorb water or urine or ammonia as a bedding material. Hemp shavings are considered a dense litter type because of their higher volume-based moisture binding capacity even better than wood shavings and straw (Table 7). It provides an excellent urine filtering as it remains dry and smooth on the surface of the bed after one week, showing a great urine filtration as urine ends up at the bottom of the stall box. Therefore, to allow the surface of the bed to always remain perfectly dry, it is necessary to distribute a certain thickness of litter on the bottom of the bed. The relative ammonia absorption reported for hemp is greater than wood chips and straw (Table 8). The absorption capacity showed by hemp reduces the need of litter replacement as it remains longer in the stable, saving working time and making daily litter care easy (Airaksinen et al. 2001; Häubermann et al. 2002; Airaksinen 2006; Russo et al. 2008; Bambi et al. 2018; Ely et al. 2022; Visković et al. 2023).

Table 6. Water absorption parameters of different bedding materials

Bedding material	WHC (1kg bed/10L water) at room T°	Water binding capacity (% DM)	Moisture binding capacity (Wt. %)	WHC (%)	WHC (kg/kg)
Hemp	22.5 ± 0.53			43	
Hemp shavings			325		
Hemp straw					1.66
Hemp shives		356.2±8.99			
Peat moss	14.8 ± 0.97			66	
Linen	19.0 ± 1.40	330.3 ± 10.96		51	
Sawdust	9.7 ± 0.28			100	3.19
Shredded newspaper	27.2 ± 3.43	392.3 ± 17.17		36	
Wood chips	31.9 ± 3.72				1.29
Wood shavings		315.9 ± 18.47		30	
Wheat straw		320.8 ± 12.41			3.32
Cereal straw			305		

WHC: Water holding capacity; Airaksinen et al. 2001, Fleming et al. 2008, Haubermann et al. 2002, Ferraz et al. 2020

Table 7. Ammonia adsorption and liquid absorption capacity of bedding materials

	Quality bedding material			
	Very Good	**Good**	**Quite good**	**Poor**
Liquid absorption capacity	Peat Sawdust	Hemp Linen Shredded newspaper	Wood shavings	Straw
Ammonia adsorption capacity	Peat	Hemp Linen Shredded newspaper Sawdust	Wood shavings	Straw

Airaksinen 2006

Table 8. Relative ammonia absorption of different bedding materials

Bedding material	Relative ammonia absorption at 17.4°C
Hemp	60
Peat moss	100
Linen	76
Sawdust	64
Shredded newspaper	52
Wood chips	44 ± 11.1
Straw	4 ± 11.3

Airaksinen et al. 2001

Some other features of hemp as an animal bedding material is that it is unpalatable, completely biodegradable, controls odor and is no-allergenic. Animals will not be tempted eat hemp hurds as they are unpalatable. After used as litter, hemp can easily degrade into an excellent compost to be used as an organic fertilizer (Table 9). In addition, it has greater odor-suppressing properties than wood shavings, straw and hay. Hemp is considered a non-allergenic bedding material because is dust-free. Shredding hurd fibers creates an animal bedding that releases little dust and sometimes its process follows a dust removal step, which may support animals' respiratory health. So, it may cause less irritation than some wood fibers. In fact, hemp bedding is especially suited to horses allergic to straw. Hemp hurds can reduce microbial growth, in some species, relative to chips or shavings. Additionally, hemp is comfortable as a bed material because they are normally cut into little pieces and offers a solid base that doesn't frequently shift when put out on the ground (Russo et al. 2008; Bambi et al. 2018; Roach et al. 2019; Visković et al. 2023).

Table 9. Estimation of the decomposition of bedding material in horse manure storage and its effect on plant utilization of manure

Bedding material	Decomposition of bedding in manure storage	Plant utilization of manure
Hemp	Quite quick	Quite easy
Peat	Quick	Easy
Straw	Quite quick	Quite easy
Wood shavings	Slow	Problematic
Sawdust	Slow	Problematic
Shredded newspaper	Slow	Problematic

Airaksinen 2006

It is likely that animal bedding will remain the most important application of hemp hurds because their obtention is costly. But even though hemp bedding is generally more expensive than sawdust or wood shavings it is well accepted by farmers because of all its advantages already mentioned plus it produces less manure per year in comparison to the typical bedding materials (Table 10). In some countries the domestic supply of hemp-based bedding hasn't been available, so users have had to pay for importing the material. In Europe, the animal bedding market is not considered important, but in North America there are insufficient hemp hurds available to meet market demand. (Roach et al. 2019; Ely et al. 2022).

Table 10. Annual amount of produced bedding manure per horse when different bedding materials are used

Bedding material	Bedding manure/horse/year (m^3)
Hemp	9.1
Peat	9.8
Shredded newspaper	11.7
Wood chips	12.4
Long straw	19.5

Airaksinen et al. 2001

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Chapter 1.

Hemp (*Cannabis sativa L*.) Seed and Co-Products Inclusion in Diets for Dairy Ruminants: A Review

1.1 Abstract

Recently, hemp (*Cannabis Sativa L.*) was rediscovery as a plant that offers a wide variety of applications (textile, pharmaceuticals, construction, etc.), including also the use in animal and human nutrition. The inclusion of whole seeds and co-products obtained by processing of seeds (cake, meal, and oil) in the diets of farm animals can allow the transfer of bioactive substances to human food. Few publications are available on the use of hemp in dairy ruminants but some authors reported a positive effect on the fatty acids profile of milk and cheese with an increase of n-3 fatty acids and c9,t11 conjugated linoleic acid. The protein content, amino acids profile, and rumen undegradable protein (RUP) of hempseed and co-products of hemp appear interesting and suitable for ruminant nutrition. Negative effects of anti-nutritional factors (i.e., phytate) are not observed. However, the researches on the effects of the use of hempseed and co-products in diets for dairy ruminants do not allow to suggest optimal levels of inclusion. In addition, no data are published on the use of whole or part of the hemp plant as forage, as another possibility to use the hemp in the perspective of the circular economy.

1.2 Introduction

The consumption of animal products (meat, milk, and eggs) is growing globally mainly due to an increase in world population, greater incomes, and urbanization [1]. The growing demand for livestock products can have an undesirable impact on the environment, considering, in particular, low energy conversion ratio from feed to food and the high requirements of land and other input (i.e., water, nitrogen) to produce the feed for animals. Ruminants are animals with a lower efficiency to convert the energy of feed in food considering the losses due to rumen fermentation processes. On the contrary, ruminants play an important role in the bio-economy by converting food not edible by humans (i.e., forages, crop residues, and agricultural by-products) into high nutritional value food [2,3].

On this basis, alternative plants have been recently rediscovered and reintroduced on the agricultural surfaces by exploiting (i) their higher resistance to the adverse conditions (i.e., drought, pathogens); (ii) their role as phytoremediation and soil revitalization [4]; and (iii) their lower nutritional requirements compared to traditional sources of energy and protein in ruminant feeding (mainly corn meal and corn silage, soybean meal, etc.). The hemp plant (*Cannabis sativa L.*) is undoubtedly one of the most cultivated plants throughout history in the world.

The surface of hempseed in Europe, estimated by European Industrial Hemp Association (EIHA) [5], was about 50,081 hectares in 2018 with an increase of 3.3%, 70%, and 614% compared with 2017, 5-years average and 1993, respectively. The major producers in the world are Canada and USA with an estimated 315,000 and 1160 hectares respectively, as reported by Semwogerere et al. [6].

In the past, hemp has been cultivated primarily to obtain fibers from the stem [7,8]. The seeds have traditionally been used for therapeutic purposes and in pharmaceutics and chemistry [9], and the cannabinoid-containing flowers have been utilized for medicinal, spiritual/religious, and recreational purposes [10]. In Europe, the varieties allowed to be cultivated must be listed in the European Union (EU) Common Catalogue of Varieties of Agricultural Plant Species. The varieties must contain <0.2% delta-9-tetrahydrocannabinol (THC, in dry matter basis), which is the main psychoactive substance [11]. The interest to this plant is mainly oriented to produce seed for human and animal nutrition, shives for construction (green building) and animal bedding, and fibre for textile and paper industry ("industrial hemp"). In dairy ruminant nutrition, hempseed and derivatives (oil, cake and meal) can be used as a supplement in feed mainly as sources of essential fatty acids and essential amino acids [12].

The aim of the present review paper was to report an update of data on the chemical and nutritional characteristics of hempseed and derivatives and a state of the art on the researches on the use in dairy ruminant feeding, considering their effects on the milk yield and quality.

1.3 Chemical Composition and Nutritive Value of Hempseed and Derivatives

1.3.1 Chemical Composition and Nutritive Value of Full-Fat Hempseed

The whole (full-fat) hempseed (HS) can be used as fed in the animal feeding or after the treatments to removal lipid components using cold mechanical pressing in order to obtain hempseed cake (HC) or, less frequently, by chemical extraction using organic solvents to obtain hempseed meal (HM). Some authors use the term "hemp meal" or "hemp flour" to indicate the product obtained by the mechanical extraction because the cake is often subjected to grinding and then it is in the form of powder. In this paper, "hempseed cake (HC)" will be used for both of these products.

Hempseed varieties, which are generally used for animal nutrition, are considered THC free; however, some studies have reported traces of THC present in the seed sample probably because it was contaminated with plant debris [13].

In Table 1, data of the chemical composition of the full-fat hempseed reported in the literature are shown. The expected differences of the chemical composition in the published studies are due to the effect of variety/cultivar, preliminary treatments (i.e., decortication), different pedological and climatic situations, and agronomical practices. The ripened seed of hemp is an excellent protein source in animal feeding (on average 24.8 ± 2.0% on dry matter, DM). A similar value of crude protein (25% on DM) for hempseed was reported by European Food Safety Authority (EFSA) [11]. Considering other protein sources, largely diffused in animal feeding, the hempseed can be located as an intermediate crude protein (CP) source between soybean (39.2 ± 5.4% on DM) and sunflower seeds (19.2 ± 4.2% on DM) [14]. The average percentage of lipids in hempseed is very high and results in 30.9 ± 4.2% on DM. Lower values were found by Arango et al. [15], considering six different varieties, cultivated in the north of Italy (province of Rovigo) in 2019.

The neutral detergent fiber (NDF) content (Table 1) showed a large variability among the authors, ranging from 29.7–37.2% on DM. Only four publications reported the energy value of hempseed, resulting on average 2422 ± 97 and 946 ± 117 kJ/100 of DM respectively for gross and net energy for lactation in sheep [16].

Table 1. Chemical composition (% on DM basis) of full-fat hempseed.

References	[17] [1]	[18] [2]	[19]	[20]	[15] [3]	[9]	[21] [4]	[22] [5]	[23]	Mean ± SD
DM, %	91.0	88.2	91.2	93.4	94.8	93.5	89.7	93.8	91.3	91.9 ± 2.2
Ash	4.9			5.8	4.7	6.0		5.5		5.4 ± 0.6
Crude protein	25.3	25.7	24.9	24.9	21.8	26.5	21.3	25.6	27.4	24.8 ± 2.0
Ether Extract	33.9	31.6	32.7	33.2	23.5	38.0	27.7	29.2	28.4	30.9 ± 4.2
Total dietary fiber (TDF)						27.6				
NDF	37.0	33.4	29.7	37.2				35.7	33.4	34.6 ± 3.1
ADF [6]		23.2	21.3					27.8	23.3	24.1 ± 3.3
Gross Energy [7]				2490		2353				2422 ± 97
Net Energy [7,8]		1029	863							946 ± 117

[1] Decorticated seed, [2] Cultivar Armanca, [3] Average of 6 cultivars, [4] Cultivar Jubileu, [5] Average of 10 cultivars, [6] Acid detergent fiber, [7] Energy is expressed as kJ per 100 g of DM, [8] Net Energy for lactation (sheep), estimated according to INRA [16].

Identification and characterization of hempseed proteins showed that edestin, rich in valuable amino acids (as glutamic acid and aspartic acid), is the main protein component in isolate hempseed protein fraction [24]. Another protein structure, rich in methionine and cystine, was found in hempseed and, subsequently, characterized as an albumin protein family member [25]. Callaway et al. [9] reported, for the first time, the amino acidic profile of hempseed (cultivar Finola) in comparison with the other protein sources. The composition of essential amino acids of hempseed, soybean, and rapeseed [9] compared with the reference pattern recommended by FAO/WHO/UNU [26] in human nutrition, is presented in Figure 1. The contents of the sulphur-containing amino acids and histidine of hempseed are very similar to those of the other two protein sources. Only levels of lysine, threonine, and tryptophan are lower in hempseed compare to soybean and rapeseed.

Considering the reference pattern of FAO/WHO/UNU [26] for adults, the limiting amino acid of hempseed is lysine (chemical score: 0.23).

Hempseed contains anti-nutritional compounds that reduce the absorption of protein and micronutrients. In particular, the phytate (inositol hexaphosphate) content in the seeds and cake of hemp can be over 5% [27]. The absorption of mineral elements and vitamins can be reduced by phytic acid, during the gastrointestinal passage, producing an insoluble final product [28]. Therefore, an additional amount of microelements is needed to maintain the efficiency of the metabolic processes that support growth, development, and a correct functioning of the organism [29]. Reggiani and Russo [30] observed that the replacement of 6.4% (on DM basis) of corn and soybean with hempseed or flax, maintaining the diets isonitrogenous, can increase the availability of iron in Alpine lactating goats. The authors speculate that some substances (i.e., inulin) contained in hemp or flax seeds can stimulate the absorption of iron.

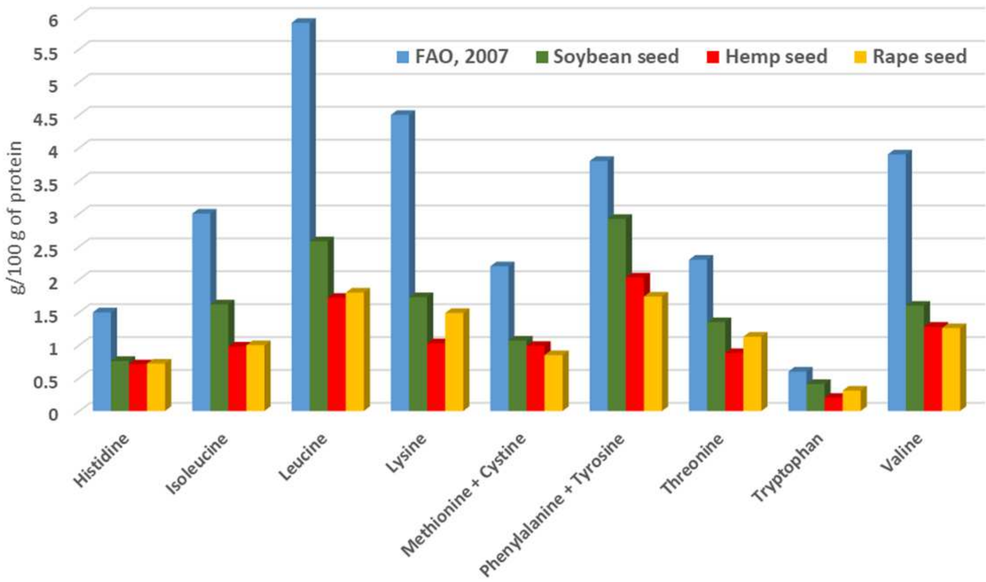

Figure 1. Content of essential amino acids (g/100 of protein) in soybean, hemp, and rape seed [9] in comparison with the reference pattern of FAO/WHO/ONU [2] for human nutrition.

1.3.2 Chemical Composition and Nutritive Value of Hempseed Meal

After oil extraction, the hempseed cake (HC) can be used as optimal protein source for dairy ruminants. The chemical composition of HC has been reported by several authors (Table 2). As expected, crude protein content increases in HC in comparison with hempseed, and the average value is 34.3% on DM. As other oilseeds, cold mechanical extraction of seed produces a cake that is higher in oil compared with the corresponding chemically obtained meals. The method of extraction is very important not only to obtain an oil of good quality but also to have a high oil yield [31,32]. The percentage of residual oil in the cake is 11.7–12.5% on DM for all authors; only Silversides [20] found a higher concentration of lipids (17.9% on DM). The content of fiber fractions increases in the hemp cake in respect to hempseed (about +27% and +42% for NDF and ADF, respectively).

Table 2. Chemical composition (% on DM basis) of hempseed cake (obtained by cold oil pressing).

References	[9] [1]	[18] [2]	[33] [1]	[20] [3]	[15] [4]	[34]	Mean ± SD
DM	94.4	89.4	93.7	91.4	93.8	89.2	92.0 ± 2.3
Ash	7.6		6.7	7.9	5.8	6.5	6.9 ± 0.9
Crude Protein	35.5	33.4	34.4	33.6	31.4	37.7	34.3 ± 2.1
Lipids	11.8	11.7	12.4	17.9	12.5	9.6	12.7 ± 2.8
NDF		43.6	39.3			44.2	43.1 ± 2.6
ADF		36.2	32.1				34.2 ± 2.9
Gross Energy [5]	1801			2319			2060 ± 366
Metab. Energy [5,6]			950			1256	1103 ± 216
Net Energy [5,7]		761					

[1] Cultivar Finola, [2] Cultivar Armanca, [3] Cultivar Unika-b, [4] Average of 6 cultivars, [5] Energy is expressed as kJ per 100 g of DM, [6] Metabolisable Energy, estimated according to Axelsson, 1941 [35], [7] Net Energy for lactation (sheep), estimated according to INRA [16].

1.3.3 Chemical Composition and Nutritive Value of Hempseed Oil

The quality of the oil obtained by chemical extraction is lower than that obtained by mechanical extraction. For this reason, hempseed meal is used mainly in the industrial processes (lubricants, detergents, paints).

In Table 3 is shown the fatty acid profile of whole hempseed (HS), hemp cake (HC) and hemp oil (HO) reported in the literature in order to compare the composition of fatty acids (FA) in the different products.

The contents of saturated FA (SFA) were very variable within the different products (from 8.2 to 14.5; from 7.7 to 13.1, and from 7 to 11.6 % of total FA for whole hempseed, cake and oil, respectively). In hempseed products, palmitic (C16:0) and stearic (C18:0) acids represent the higher percentages of SFA (on average 65% and 24% respectively). As known, these long-chain SFA, if consumed in excess, have been associated with increased cardiovascular disease risk in the human population [36,37].

The average values of the percentages of mono-unsaturated FA (MUFA) in the three products are very similar (13.4, 12.5, and 13.0% of total FA for hemp seed, cake, and oil, respectively). However, the variability within each group is very high, especially in oil (from a minimum of 9.0 to 20.7% of total FA). The oleic acid (C18:1) represents a very high percentage (from 93 to 98% of total MUFA).

As shown in Table 3, the sum of polyunsaturated fatty acids (PUFA) of hemp products is around 75%, and this value is reported as a mean by other authors [9,38,39]. The differences of single PUFA among the three products are very small but, within group, the variability is high, especially for alpha linolenic acid (ALA) in whole hempseed (from 12.98 to 22.4% of total FA). Over 70% of the PUFA are linoleic acid (LA; 18:2 n-6) and ALA (18:3 n-3) [40]. Small amounts of gamma-linolenic acid (GLA; 18:3 n-6) and stearidonic acid (SDA; 18:4 n-3), the biological metabolites of LA and ALA, respectively, were found by some authors (on average 4 and 2% of total FA) [41]. In all publications, the n-6/n-3 ratio is lower than 5:1, which has been claimed as ideal for humans [41,42].

Table 3. Fatty acid profile (% of total FA) of lipids contained in whole hempseed (HS), hemp cake (HC) and hemp oil (HO).

References	**[43]**	**[43]**	**[17]**	**[18]**	**[19]**	**[15]**	**[18]**	**[44]**	**[15]**	**[9]**	**[45]**	**[46]**	**[47]**	**[15]**
Products	HS [1]	HS [2]	HS	HS [3]	HS	HS [4]	HC [5]	HC	HC [4]	HO [5]	HO	HO	HO	HO
C12:0					0.26	0.11			0.09					
C14:0	0.12	0.07			0.04	0.19		0.07	0.17		0.03		0.03	0.08
C16:0	7.27	7.37	6.47	6.2	5.89	9.10	9.3	4.46	8.77	5.0	6.07	7.9	6.54	7.46
C18:0	3.01	2.67	2.87	2.1	2.05	2.72	3.8	1.76	2.51	2.0	2.38	2.70	2.73	2.50
C20:0	3.93	4.40	0.91			0.91		0.71	0.74		0.87	0.8		1.53
C22:0								0.38			0.34			
C24:0								0.22			0.17			
Others SFA	0.12		0.68					0.06						
Total SFA	14.45	14.51	10.93	8.3	8.24	13.03	13.1	7.66	12.28	7.0	9.86	11.4	9.3	11.57
C16:1	0.24	0.32			0.15	0.17		0.15	0.14		0.14	0.2		0.14
C18:1n-7								0.85						
C:18:1n-9	13.14	13.57	12.06	9.5	10.11	16.14	13.1	8.27	13.83	9.0	10.26	20.3	10.91	13.45
C20:1n-9	0.85	0.93	0.42		0.62	0.49		0.46	0.52		0.40	0.4		
C22:1n-9			0.02		0.28			0.00			0.03			
C24:1n-9								0.10						
Other MUFA	0.46	0.84	1.11		0.15			0.32			0.22			
MUFA	14.45	15.34	13.61	9.5	11.16	16.33	13.1	10.00	14.35	9.0	10.91	20.7	10.91	13.45
C18:2 n6	55.34	55.15	56.2	56.1	56.50	55.59	52.5	59.52	56.98	56.0	55.75	51.3	55.78	54.59
C18:3n-3	15.15	14.74	15.25	22.4	21.15	12.98	19.1	15.85	14.62	22.0	17.37	15.70	20.65	15.83
C18:3n-6			2.97	3.7		1.45	2.2	4.52	1.60	4.0	4.65	0.00		
C18:4n-3			0.89							2.0	1.48			
C20:2n-6								1.38						0.19
C20:3n-3	0.45	0.40						0.05						
C20:5n-3								0.16						
Other PUFA	0.16	0.14			0.05			0.10						
Total PUFA	71.10	70.15	75.5	82.2	77.7	70.02	73.8	81.58	73.2	84	79.25	67	76.43	70.61
n-3 PUFA	15.60	14.89	16.14	22.4	21.15	12.98	19.1	16.06	14.62	24	18.85	15.7	20.65	15.83
n-6 PUFA	55.34	55.15	59.17	59.8	56.5	57.04	54.7	65.52	58.58	60	60.40	51.3	55.78	54.78
n-6/n-3 ratio	3.55	3.70	3.67	2.67	2.67	4.39	2.86	4.08	4.01	2.50	3.20	3.27	2.70	3.36

[1] Cultivar Fedora 17, [2] Cultivar Ferimon, [3] Cultivar Armanca, [4] Average of 6 cultivars, [5] Cultivar Finola.

1.4 Use of Hempseed and Derivatives in Dairy Ruminants

1.4.1 Use of Hempseed and Derivatives in Dairy Cows

The interest in the development of different feeding strategies to improve the chemical nutritional properties of dairy milk and milk products, assuming that nutrition can influence milk composition in ruminants, has grown in the last years [48–53]. Considering the high level of n-3 and n-6 fatty acids and the optimal n6/n3 ratio in hempseed, an increase of these PUFA could be expected in milk and derivatives. However, no papers are available to date on the effects of hempseed cake inclusion in the diet of dairy cows on fatty acid profile of milk and derivatives.

There is only one published paper on the use of hempseed or its co-products in dairy cows. Karlsson et al. [33] evaluated the effects of increasing the proportion of hempseed cake (HC) in the diet of dairy lactating cows on milk production and composition. Four experimental diets (based on a ratio of 494:506 g/kg of DM between silage and concentrate mixture) were formulated to contain increasing concentrations of HC: 0 (HC0), 143 (HC14), 233 (HC23) or 318 (HC32) g/kg of DM. No effects in DM intake but significant linear increases in CP, fat, and NDF intakes were observed with the increase of the proportion of HC in the diets. Increasing HC dietary levels resulted in significant quadratic effects on the milk yields and energy-corrected milk, with the highest value for the HC14 group (Table 4).

The milk protein and fat percentage decreased linearly ($p < 0.05$) with the increasing of HC in the diet. Furthermore, there was a significant ($p < 0.001$) linear increase in milk urea concentrations with the enhancement of HC inclusion due to the increase of CP intakes. A linear decrease in CP efficiency (milk protein yield/crude protein intake) was also observed. The best and maximum suggested level of HC inclusion in this experiment was 143 g/kg DM.

Table 4. Effect of hempseed cake (HC) on milk yield and composition [33].

Groups	HC Dosage (% DM)	Milk Yield (kg/d)	Milk Protein (%)	Milk Fat (%)	Milk Urea (mmol/l)	Protein Efficiency [1]
Control	0	25.2	3.63	4.31	2.7	0.29
HC14	14.3	28.7	3.61	4.21	3.7	0.26
HC23	22.3	26.8	3.49	4.07	4.4	0.22
HC32	31.8	26.8	3.40	3.89	5.1	0.22
p-value [2]		0.022	0.028	n.s.	<0.001	0.009

[1] Milk protein yield/crude protein intake, [2] n.s. = not significant

Mustafa et al. [54] determined the DM and CP in situ degradability in two nonlactating rumen fistulated cows of four different protein sources (hemp, borage, canola, and heated canola meals). The results showed that hemp meal resulted in an excellent natural source of rumen undegradable protein (RUP) (774 g/kg of CP), equivalent to heat-treated canola meal but higher than borage and canola meals.

In conclusion, further studies are required to determine the effects of including HC in dairy rations, suggesting to maintain the diets as isoenergetic and isonitrogenous, modifying the proportion of the other ingredients. In addition, the nutritional value of milk and derivatives (i.e., fatty acids profile, vitamins, bioactive substances) could be determined to know the possible nutraceutical effects of hempseed meal.

1.4.2 Use of the Hempseed and Derivatives in Dairy Ewes

Ewes milk would naturally have a high content in substances beneficial to human health, such as n-3 fatty acids (FAs) and conjugated linoleic acid (CLA). The n-3 FAs, especially eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3), can reduce the risk of cardiovascular diseases and in experimental animals, c9,t11 CLA has been proved to possess anticancer and anti-atherosclerotic effects, as well as anti-obesity activities [55]. As above reported, to increase the concentration of PUFA in milk, different sources of unsaturated plant lipids (i.e., linseed, soybean, safflower, and sunflower) could be included successfully in the diet [56,57]. The disadvantage of milk enriched with PUFA is the possibility of oxidation owing to its high content of double-bonded molecules, which are prone to oxidation [58]. The delicate balance between anti- and pro-oxidative processes in milk is influenced by different factors such as ruminant feeding, degree of unsaturated fatty acids, contents of transition metal ions and antioxidants such as tocopherols and carotenoids [59].

In this context, Mierlita et al. [18] carried out an experiment using 30 Turcana dairy sheep divided into three groups consisting of a control diet (C diet) based on hay and supplemented by mixed concentrates and two experimental diets designed to provide the same amount of fat using hempseed (180 g/d) (HS diet) or hempseed cake (480 g/d) (HC diet). The three diets were isoenergetic and isonitrogenous, and the two diets with hemp had the same amounts of PUFA. Hemp (HS and HC diets) increased milk yield and milk fat content but decreased milk lactose (Table 5). The hemp feeding increased the PUFA content (especially n-3 fatty acids) in ewes milk and improved the n-6/n-3 ratio. Total CLA content doubled in the milk of the ewes that received hempseed and increased by 2.4 times with the hemp cake inclusion (Table 6). The alpha-tocopherol and antioxidant activity increased using hempseed in the diets, reducing the risk of lipid oxidation in raw milk.

Traditionally, ewes on farms are fed indoor or often on part-time grazing during much of the lactation period. During this period, the c9,t11 CLA and n-3 FA contents in milk are lower than that observed during grazing [60]. Mierlita et al. [19] studied the effects of a part-time grazing system or indoor feeding and the supplementation of hempseed in the diet on milk yield and quality, FA profile, and health lipid indices in the raw milk of dairy ewes. Forty ewes were used in this 10-week experiment and were divided into four groups: indoor feeding system with and without hempseed and part-time grazing with and without hempseed. Feeding with the addition of hempseeds significantly increased milk fat content and fat yield (Table 5). Milk protein content was not affected by dietary treatments. Hempseed supplementation increased the content of total PUFA, n-3 and n-6 fatty acids. In the indoor feeding system, the CLA content doubled with the hempseed addition (1.13 vs. 2.29% of total FA) but increased also in the milk of grazing sheep (+37%) (Table 6). As known, the availability of precursors (i.e., linoleic acid) for ruminal biohydrogenation and synthesis of CLA is high at pasture when the animals were fed fresh forage [61].

Table 5. Effect of hempseed and derivatives on the chemical composition of ewe milk.

References	Treatment [1]	Dosage (% on DM)	Milk Yield (g/d)	Milk Protein (%)	Milk Fat (%)	Lactose (%)
[18]	CTR	0	728	5.61	7.42	5.20
	HS	6.7	781	5.60	8.12	5.10
	HC	22.6	767	5.62	7.97	4.85
		p-value [2]	<0.05	n.s.	<0.01	<0.05
[19]	I	0	669	5.78	7.45	5.20
	I+ HS	8.3	686	5.61	8.36	5.14
	PTG	0	770	6.11	7.39	5.02
	PTG + HS	8.3	784	6.15	7.98	5.09
		p-value [2,3]	n.s.	n.s.	<0.01	n.s.
[50]	CTR	0		5.25	6.40	4.69
	HS	5.0		5.17	5.96	5.84
		p-value [2]		n.s.	n.s.	<0.01

[1] CTR = control; HS = hempseed; HC = hemp cake; I = indoor feeding system; PTG= part-time grazing feeding system; [2] n.s. = not significant, [3] *p*-value: effect of HS supplementation.

Table 6. Effect of hempseed and derivatives on the fatty acid profile (% of total FA) of ewe milk.

References	Treatment [1]	Dosage (% on DM)	PUFA [2]	n-3	n-6	n6/n3	CLA
[18]	CTR	0	6.98	1.99	3.81	1.91	1.18
	HS	6.7	9.85	3.34	4.12	1.23	2.39
	HC	22.6	10.60	2.94	4.35	1.48	2.81
		p-value [3]	<0.001	<0.01	n.s.	<0.01	<0.01
[19]	I	0	5.63	1.31	0.30	5.63	1.13
	I+ HS	8.3	7.92	1.67	0.35	7.92	2.29
	PTG	0	7.40	2.06	0.39	7.40	2.12
	PTG + HS	8.3	9.11	2.09	0.56	9.11	2.90
		p-value [3,4]	<0.001	<0.01	<0.01	<0.01	<0.01

[1] CTR = control; HS = hempseed; HC = hemp cake; I = indoor feeding system; PTG= part-time grazing feeding system; [2] PUFA = polyunsaturated fatty acid. [3] n.s. = not significant, [4] *p*-value: effect of HS supplementation.

Ianni et al. [50] evaluated the effects of a diet enriched with hempseed (5% on DM basis) on the chemical characteristics of milk and cheese from 32 half-bred dairy ewes. The enrichment of dairy ewes' diet with HS increased the lactose concentration from 4.69% to 5.84% but no significant differences were observed in milk fat, protein, casein, and urea (Table 5). In addition, no changes were detected in total fat, protein, and ash in derived cheeses. During the experiment reported by Ianni et al. [50], the first RNA sequencing of the whole blood transcriptome on ewes of the two experimental groups (0 and 5% of hempseed on DM) was described by Iannaccone et al. [13]. Hempseed supplementation positively affects the pathways related to energy production in lactating ewes. This condition could also be potentially beneficial to increase the resistance to adverse climatic conditions such as low temperature.

A digestibility experiment on sheep was conducted by Mustafa et al. [54] using hemp meal (5.2% of lipids on DM) at different levels of inclusion (0, 50, 100, 150, 200 g/kg of DM) in replacement of canola meal, maintaining isonitrogenous diets, based on barley. Voluntary DM intake was not affected by the hemp meal inclusion levels. Total tract DM and organic matter digestibility values were similar across treatments, suggesting that digestibility of hemp meal is equal to that of canola meal. The authors concluded that the hemp meal can be used up to 20% on DM with no detrimental effects on nutrient utilization by sheep.

1.4.3 Use of the Hempseed and Derivatives in Dairy Goats

Goat milk has high concentrations of caproic (C6:0), caprylic (C8:0), and capric (C10:0) acids, known to exhibit antiobesity and antidiabetic properties [62]. Also in dairy goats, the interest of modulating milk fat composition by diet leads to the supplementation with feed sources rich in PUFA as an efficient way to modify milk FA profile. The oils extracted by oleaginous seeds can directly affect the fatty acid composition of milk and derivatives but could also have negative effects in terms of animal health status and, in particular, on the efficiency of the rumen microorganisms.

Cozma et al. [47] have evaluated the effect of a diet supplemented with hempseed oil in Carpathian goats during 31 days of experiment. No significant changes of milk yield were observed for ewes receiving the hempseed oil supplementation. Fat content increased significantly during the trial in milk produced by goats receiving hemp oil in comparison with the control group. The increase of milk protein content, due to the hemp oil addition, is significant just until day 15 of the experiment and then values remain the same (Table 7).

Table 7. Effect of hempseed oil on milk yield and quality of goats.

References	Treatment	Dosage (% on DM)	Milk Yield (g/day)	Milk Fat (%)	Milk Protein (%)
[47]	CTR	0	1280	2.70	3.16
	Hemp Oil	4.7	1330	3.59	3.28
		p-value [1]	n.s.	<0.001	<0.05
[63]	CTR	0		3.39	
	Linseeds	9.3		3.73	
	Hempseed	9.3		3.69	
		p-value		0.013	

[1] n.s. = not significant

Cremonesi et al. [63] carried out an experiment to evaluate the effects of the inclusion of 9.3% on DM of linseed or hempseed in diet for Alpine lactating goat. The milk yield was unaffected by dietary treatment but linseed and hempseed supplementation significantly increased the milk fat content. No differences were detected in milk protein, lactose and urea concentration (Table 7).

Cozma et al. [47] found a significant increase of the PUFA concentrations (+45%) in milk produced by goats supplemented by hempseed oil, without an effect on n-3 fatty acids content. The content of cis-9, trans-11 CLA increased on average by over four times, reaching the peak during the second week of oil supplementation but then decreasing from the third week (Table 8). This transitory effect could be due to an adaptation of the rumen microorganisms to oil supplementation. Hemp oil inclusion had no effect on cholesterol concentration in milk (Table 8), even if plasma cholesterol concentration increased in the ewes fed with oil supplementation. The lack of a relationship between plasma and milk cholesterol concentration could be explained considering that a low proportion of the total milk cholesterol is derived from mammary de novo synthesis. In dairy cows, about 80% of the cholesterol in milk originates from the uptake of serum cholesterol obtained through hepatic synthesis [64]. The overall results of Cozma et al. [47] suggest, for the first time, that beneficial effects on human health can be obtained in goat milk with the inclusion of hempseed oil in the diets.

Table 8. Effect of hempseed oil (HO) on fatty acids, cholesterol and vitamin A of goat milk.

Reference	Treatment	Dosage (% on DM)	PUFA	n-3	n-6	CLA[1]	Cholesterol (mg/100 g)	Vitamin A (µg/mL)
[47]	CTR	0	5.30	1.35	2.57	0.49	14.63	0.167
	HO	4.7	7.69	1.57	2.94	2.14	11.83	0.151
		p-value [2]	<0.001	n.s.	0.10	<0.001	n.s.	n.s.

[1]cis-9, trans-11 CLA, [2] n.s. = not significant

1.4.4 Use of the Hempseed and Derivatives in Buffaloes

In several countries, buffaloes are important species for the production of milk and derivatives for human consumption. There are not any studies related to hemp as feed for improving buffalo milk. Only one published study [65] reported, in the north of Pakistan, possible exposure to delta-9-tetrahydrocannabinol (THC) by the children consuming buffaloes milk. In this region, buffaloes graze in natural pastures, where *Cannabis sativa L*. with high levels of THC grows spontaneously and higher concentrations of THC metabolites were found in buffaloes milk. As above reported, in EU countries, the hemp varieties allowed for cultivation are registered in the EU's Common Catalogue of Agricultural Plant Species and are characterized by THC value less than 0.2–0.3% [11].

EFSA [11] recommended introducing a maximum THC content of 10 mg/kg to hempseed-derived feed materials to avoid risks for human health due to consumption of food of animal origin.

1.5 Conclusions

The chemical and nutritional characteristics of hempseed and hempseed derivatives (cake, meal and oil) are updated in the first section of this review. Protein content, aminoacids profile, and ruminal undegradable protein (RUP) make these products suitable for inclusion in ruminant diets. In addition, the fatty acid composition of hemp oil allows to transfer the PUFA and, in particular, n-3 fatty acid to the milk of dairy ruminants, as reported by several authors. Up to now, few publications are available on dairy ruminants to suggest the optimal dosage of hempseed or derivatives in the different species. No information about the use of the whole plant or the different botanical fractions (i.e., leaves) is published.

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Chapter 2.

Chemical composition of whole plant, botanical fractions and by-products of ten industrial hemp varieties cultivated at the CREA-CI experimental field (Rovigo, Italy) along three consecutive years

2.1 Abstract

Hemp (*Cannabis sativa* L.) is an annual plant, globally distributed and cultivated in the past as a source of fiber. Recently, the interest in hemp cultivation has significantly increased, considering its positive environmental impact and several application fields. Over 70 hemp varieties, with different morphological, anatomical and productive characteristics are included in the EU Common Catalogue of Varieties of Agricultural Plant Species. In this experiment, ten varieties (CS, Carmaleonte, Codimono, Eletta Campana, Felina 32, Ferimon, Fibranova, Futura 75, Santhica and USO 31) obtained from outdoor cultivation at CREA-CI of Rovigo (northeast Italy) along three consecutive years (2019, 2020 and 2021) were analysed for chemical traits. The results of the partition of whole plants in the different botanical fractions showed a principal composition of stems (66.14 ± 11.25%), followed by leaves (23.07 ± 7.72%) and seeds (10.80 ± 5.75%). A dramatic increase of the proportion of stems that therefore caused the decrease of leaves and seeds was seen along the years, mostly due to environmental factors. In hemp stems, the content of NDF was very high (82.54% on DM) and the fiber resulted highly lignified. On the contrary, the leaves of hemp could be considered a good forage, with crude protein (CP) levels changing from 11.8 to 24.26% on DM and NDF contents from 21.26 to 38.25% on DM. In hemp whole seeds, the CP and lipid contents varied respectively from 14.05 to 25.18% on DM and from 3.24 to 28.70% on DM, showing a large variability among varieties and specially along the years. In animal feeding, full-fat seeds but also the products obtained by cold mechanical pressing of seeds (oil and cake) can be used. In hemp oil, the total saturated fatty acids (SFA), mainly represented by palmitic acid, varied from 5.9 to 16.4% of total FA. Over 90% of the monounsaturated FA (MUFA), which varied from 11.4 to 21.65% of total FA, was represented by oleic acid. The percentage of polyunsaturated FA (PUFA) was very high in the hemp oil and reached values close to 75% of the total FA (from 56.9 to 77.6% of total FA). Among PUFA, the linoleic acid (C18:2 n6) and alpha-linolenic acid (C18:3 n3) are the most represented FA (on average 54.9 and 14.9% of the total FA resp.). The n-6/n-3 ratio ranged from 2.61 and 7.9. In conclusion, the wide variability of the proportions and the chemical composition of the products obtained from the tested hemp varieties are mostly due to environmental factors but results showed that they are still suitable to use in animal feeding as they are a good source of fat and protein.

2.2 Introduction

Hemp (*Cannabis sativa L.*) is an annual plant, globally distributed and cultivated in the past as a source of fiber. Recently, the interest in hemp cultivation has significantly increased, considering its positive environmental impact and several application fields. Over 70 hemp varieties, with different morphological, anatomical and productive characteristics are included in the EU Common catalogue of Varieties of Agricultural Plant Species. Italy, as the fifth country in the EU leading the cultivation of hemp is currently contributing to the developing of this multi-purpose crop. The starting point in order to get to know any new crop is to assess the agronomical needs and cultivation requirements. The following aspect to focus on should be the nutritional composition of the final products to satisfy the consumers. It is well known that climate conditions affect agronomic and nutritional traits of any growing crop. Considering that in recent years the temperature and humidity are variable factors because of climate change, the importance of continuing evaluating the chemical composition of hemp is highly recommended. Therefore, the aim of the study was to assess the botanical composition of hemp as well as the chemical characterization of the whole plant, fractions and by-products (oil and cake) of ten varieties along three consecutive years (2019,2020 and 2021).

2.3 Materials and Methods

2.3.1 Materials

Ten hemp varieties (CS, Carmaleonte, Codimono, Eletta Campana, Felina 32, Ferimon, Fibranova, Futura 75, Santhica and USO 31) were used. The outdoor cultivation took place at CREA-CI Rovigo (northeast Italy) during the years 2019, 2020 and 2021. For year 2019, only 7 varieties were cultivated. For years 2020 and 2021, the ten varieties were cultivated. For the three years, the sowing was mostly done in April and the harvest in September-October after 143 days of cultivation in average (Table 1). The climate conditions of the cultivation field along the three years were monitored and are shown in Figures 1 to 3. The sowing was done with a density of 60 kg/ha and a distance between rows of 25 cm. A fertilization with nitrogen post emergency of 40 kg/ha was done. The whole cultivation was done mostly without irrigation, but one irrigation was done when necessary. The harvest was done mechanically.

Table 1. Hemp varieties and cultivation dates along three years

Variety	Seeding			Harvest			Days		
Year	2019	2020	2021	2019	2020	2021	2019	2020	2021
CS	19-Apr	23-Apr	26-Apr	27-Sep	7-Oct	11-Oct	161	167	168
Carmaleonte	19-Apr	23-Apr	26-Apr	16-Sep	16-Sep	15-Sep	150	146	142
Codimono	19-Apr	23-Apr	26-Apr	16-Sep	16-Sep	24-Sep	150	146	151
Eletta Campana		23-Apr	26-Apr		7-Oct	11-Oct		167	168
Felina32	4-Jun	23-Apr	26-Apr	20-Sep	16-Sep	8-Sep	108	146	135
Ferimon	4-Jun	23-Apr	26-Apr	20-Sep	31-Aug	18-Aug	108	130	114
Fibranova		23-Apr	26-Apr		7-Oct	11-Oct		167	168
Futura75	19-Apr	23-Apr	26-Apr	16-Sep	16-Sep	22-Sep	150	146	149
Santhica27		23-Apr	26-Apr		16-Sep	8-Sep		146	135
USO31	4-Jun	23-Apr	26-Apr	20-Sep	31-Aug	4-Aug	108	130	100

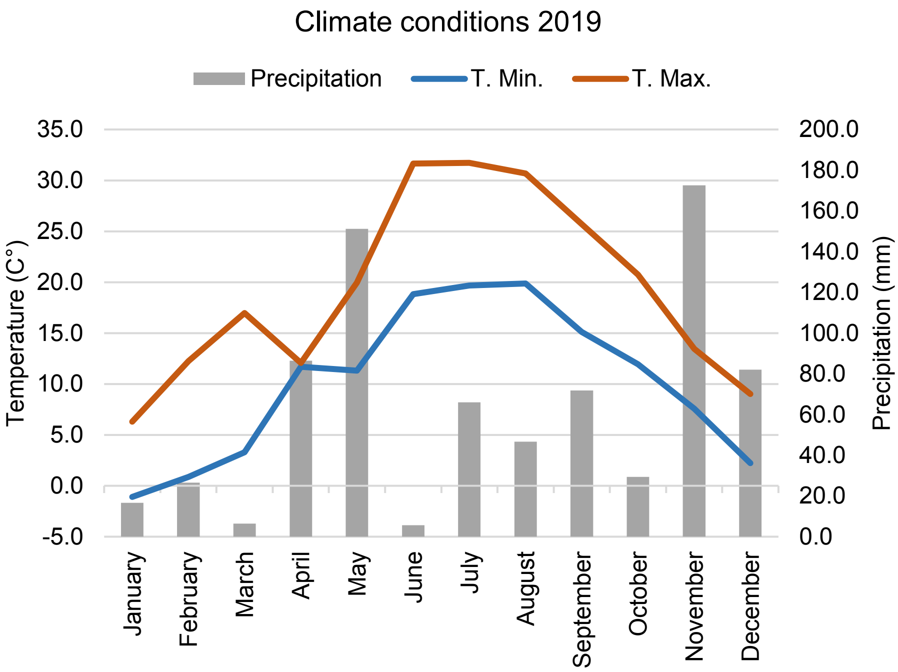


Figure 1. Climate conditions (temperature and precipitation) at the hemp field in year 2019

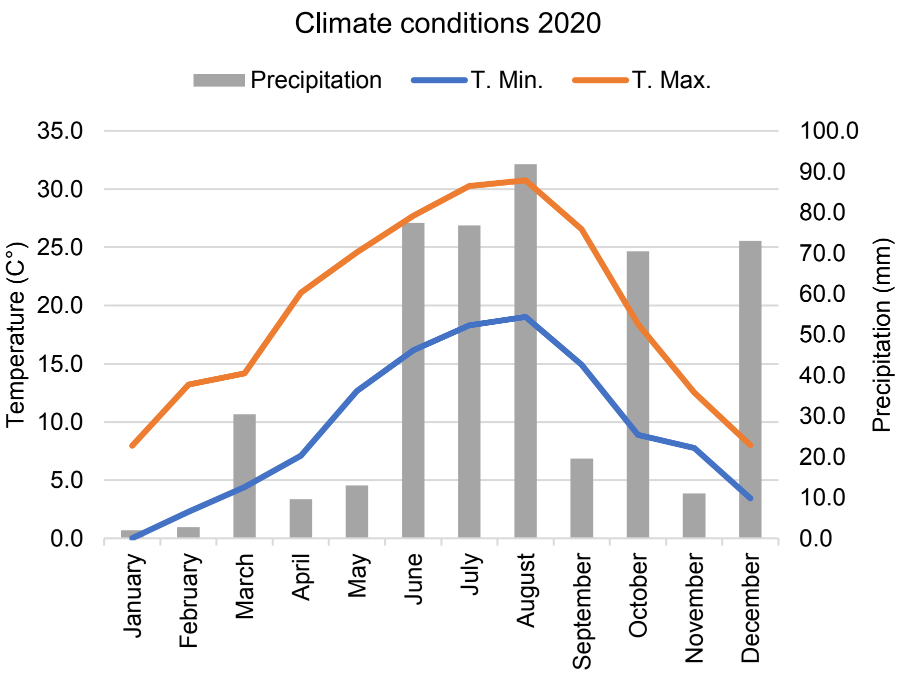


Figure 2. Climate conditions (temperature and precipitation) at the hemp field in year 2020

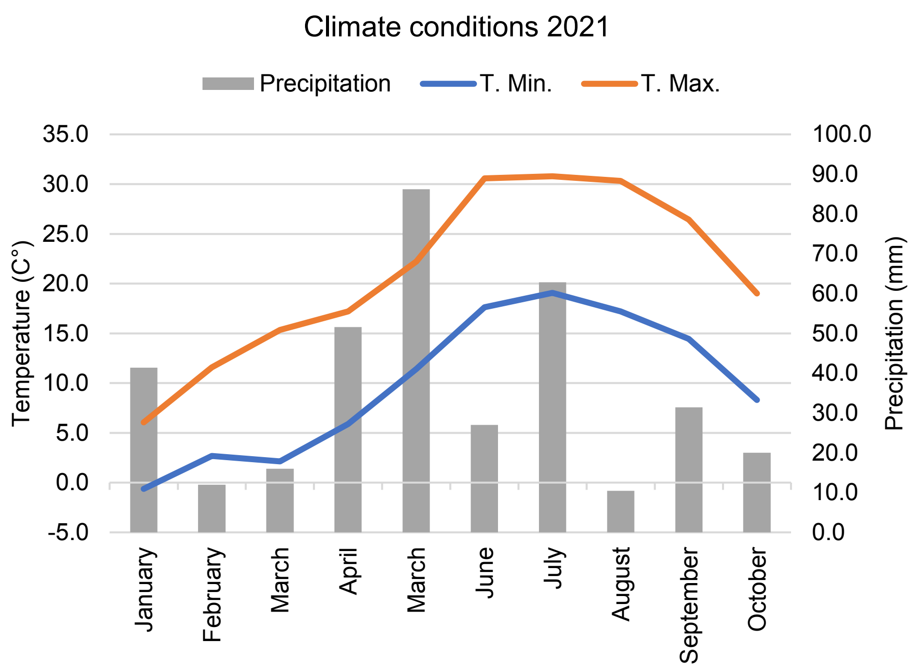


Figure 3. Climate conditions (temperature and precipitation) at the hemp field in year 2021

2.3.2 Sample preparation

For botanical fractions, three whole plants from each variety and year were taken. The separation of stems, leaves and seeds was done manually and then dried in an oven at 105°C for 48 hours. All the fractions were weighed fresh and reported as percentage of the whole plant in dry matter.

For chemical composition, three whole plants and the botanical fractions were dried in an oven at 105°C for 48 hours. Then, grounded for chemical analyses.

For fatty acid composition, 250g of seeds from each variety and year were submitted to cold mechanical pressing in order to obtain oil and cake. Prior to pressing, seed impurities were removed manually.

2.3.3 Method of analysis

The chemical analyses were done at the LaChi Lab of the University of Padova. Samples were analysed for moisture content, ash, fat, crude protein (CP), fiber, neutral detergent fiber (NDF), acid detergent lignin (ADL) and acid insoluble ash (AIA) using the official Methods of Association of Official Agricultural Chemists (AOAC) International.

Samples of seed, oil and cake were subjected to analysis of fatty acids (FA) profile, after accelerated solvent extraction (ASE 200, Dionex Corp., Sunnyvale, CA) by gas-chromatographic way. The concentration of each fatty acid was expressed as g/100g, considering 100g as the total of areas of all FAMEs identified.

2.4 Results and Discussion

2.4.1 Botanical composition of hemp plant

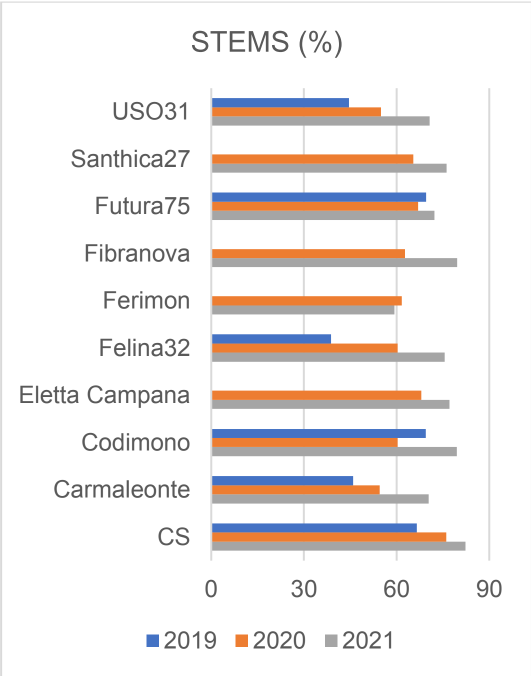
Hemp plant is mostly composed by stems (66.14 ± 11.25%), followed by leaves (23.07 ± 7.72%) and seeds (10.80 ± 5.75%) (Table 2). The proportion (%) of stems (Figure 4a) in all varieties increased along the years, showing in average 55.82, 63.10 and 74.31 for 2019, 2020 and 2021 respectively. Consequently, the proportion of leaves (Figure 4b) and seeds (%) (Figure 4c) in all varieties decreased along the years on this study. Leaves got an average of 27.12, 25.79 and 18.83 and seeds 17.08, 11.11 and 6.86 for 2019, 2020 and 2021 respectively. The large variation in seasonal temperature and specially rain fall might have been the two major reasons contributing to the increased proportion of stems and therefore reduction in seed content along the three years of study.

2.4.2 Chemical composition of hemp whole plant and botanical fractions

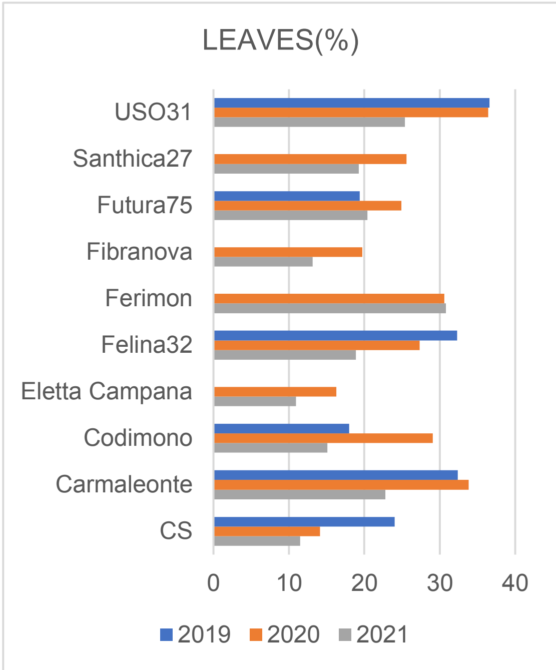
Parameters such as dry matter, ash, crude protein, fat, crude fiber, NDF, ADL and AIA are shown in Table 3. In general, the results are consistent with previous studies (Callaway 2004; Gibb et al. 2005; Silversides et al. 2005; Hessle et al. 2008; Karlsson et al. 2010; Vonapartis et al. 2015; Mierlita 2016; Wang et al. 2017; Mierlita 2018; Habenau et al. 2018). Crude protein content (% DM) was higher in hemp cake, it ranged from 5.64 to 15.45 in whole hemp plant, 1.87 to 5.32 in the stem, 11.8 to 24.26 in the leaves, 14.05 to 25.18 % in the seeds and from 13.75 to 33.83 in the cake. The varieties CS and Eletta Campana had shown the highest protein content for seeds and cake than the others. Fat content (% DM) ranged from 1.80 to 8.38 in whole hemp plant, 0.50 to 1.79 in the stem, 3.89 to 12.17 in the leaves and, 3.24 to 28.70 in seeds and 6.03 to 26.62 in the cake. The fat content was higher in hemp cake, closer to the results presented by other authors. The NDF was the most abundant fraction of the whole plant and stems of hemp.

Table 2. Botanical fraction proportions (%) of ten hemp varieties along three consecutive years

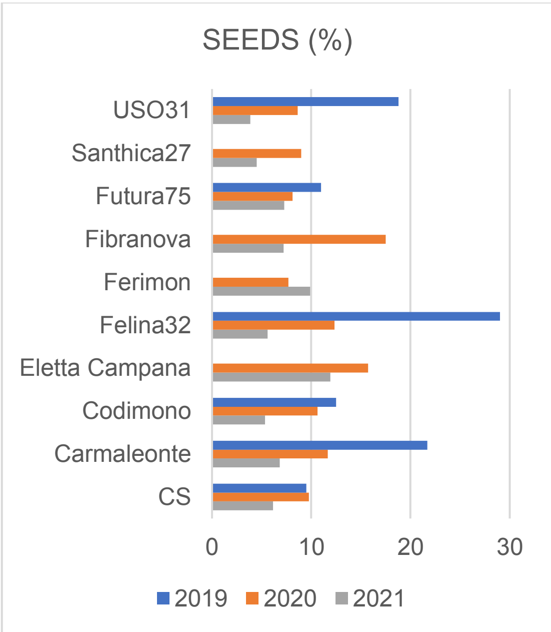
Variety	Stems (%)				Seeds (%)				Leaves (%)			
Year	2019	2020	2021	Mean	2019	2020	2021	Mean	2019	2020	2021	Mean
CS	66.50	76.12	82.34	74.99	9.50	9.78	6.15	8.47	24.00	14.11	11.51	16.54
Carmaleonte	45.90	54.52	70.37	56.93	21.70	11.66	6.84	13.40	32.40	33.82	22.79	29.67
Codimono	69.50	60.30	79.55	69.78	12.50	10.64	5.35	9.50	18.00	29.06	15.10	20.72
Eletta Campana	-	67.95	77.13	72.54	-	15.74	11.91	13.83	-	16.31	10.96	13.63
Felina32	38.80	60.33	75.52	58.22	29.00	12.35	5.60	15.65	32.30	27.32	18.88	26.17
Ferimon	-	61.72	59.32	60.52	-	7.68	9.88	8.78	-	30.60	30.80	30.70
Fibranova	-	62.76	79.62	71.19	-	17.50	7.22	12.36	-	19.75	13.16	16.45
Futura75	69.60	66.97	72.30	69.62	11.00	8.11	7.28	8.79	19.40	24.93	20.42	21.58
Santhica27	-	65.42	76.23	70.83	-	9.00	4.51	6.76	-	25.58	19.25	22.42
USO31	44.60	54.95	70.75	56.77	18.80	8.62	3.86	10.42	36.60	36.43	25.40	32.81
Mean	55.82	63.10	74.31	66.14	17.08	11.11	6.86	10.80	27.12	25.79	18.83	23.07



(a)



(b)



(c)

Figure 4. Proportions (%) of hemp botanical fractions: Stems (4a), Leaves (4b) and Seeds (4c)

Table 3. Chemical composition of hemp plant, botanical fractions and hempseed cake of ten hemp varieties along three consecutive years

	CS	Carmaleonte	Codimono	E. Campana	Felina32	Ferimon	Fibranova	Futura75	Santhica	USO31	Mean
Whole Plant											
Dry Matter											
2019	92.44	92.51	93.13	-	92.72	-	-	92.90	-	92.07	92.63
2020	93.37	93.61	93.17	93.07	92.95	93.09	92.89	93.23	92.89	92.88	93.11
2021	93.59	92.63	92.67	93.35	92.59	93.70	92.87	92.73	92.09	92.45	92.87
Mean	93.13	92.92	92.99	93.21	92.75	93.40	92.88	92.95	92.49	92.47	92.92
Ash											
2019	7.42	8.85	5.65	-	11.22	-	-	7.94	-	11.48	8.76
2020	4.25	8.81	5.77	4.25	6.52	8.69	6.44	4.67	6.04	7.08	6.25
2021	3.65	6.37	4.98	5.25	7.62	4.62	6.99	6.62	5.66	8.65	6.04
Mean	5.10	8.01	5.47	4.75	8.46	6.66	6.72	6.41	5.85	9.07	6.65
Crude Protein											
2019	12.77	12.22	6.23	-	12.94	-		7.37	-	15.45	11.16
2020	9.29	10.60	10.75	9.23	9.98	11.14	11.01	8.08	9.72	9.72	9.95
2021	5.64	6.81	7.30	10.60	10.60	6.33	10.11	9.06	6.38	11.29	8.41
Mean	9.23	9.88	8.09	9.91	11.17	8.74	10.56	8.17	8.05	12.15	9.60
Fat											
2019	8.18	8.38	6.29	-	8.20	-	-	6.53	-	7.90	7.58
2020	2.93	-	2.95	3.89	2.98	3.08	4.52	3.10	1.80	2.04	4.23
2021	2.81	3.09	2.99	6.77	4.95	4.29	3.32	3.92	2.29	3.52	3.79
Mean	4.64	8.83	4.08	5.33	5.38	3.69	3.92	4.51	2.04	4.49	4.69
Crude Fiber											
2019	38.17	40.40	46.59	-	32.74	-	-	43.12	-	31.33	38.73
2020	48.17	41.97	43.98	46.71	41.95	42.73	44.93	50.25	47.06	39.21	44.70
2021	52.43	49.67	51.11	47.42	44.27	53.24	40.82	50.11	52.49	42.51	48.41
Mean	46.26	44.01	47.23	47.07	39.65	47.99	42.88	47.83	49.78	37.68	45.04
NDF											
2019	59.68	59.89	69.49	-	51.53	-	-	64.95	-	50.23	59.30
2020	70.66	54.93	67.98	70.43	63.98	59.44	67.41	69.87	65.87	67.34	65.79
2021	77.18	74.83	76.72	60.96	69.13	76.88	59.45	75.87	74.40	65.06	71.05
Mean	69.18	63.22	71.40	65.70	61.55	68.16	63.43	70.23	70.13	60.88	66.39
ADF											
2019	43.95	46.13	54.76	-	38.45	-	-	50.44	-	36.59	45.05
2020	54.16	41.16	51.95	53.44	48.35	45.04	51.92	54.60	50.56	52.05	50.32

continue											
2021	60.22	59.30	60.75	46.42	54.82	61.37	46.48	60.02	58.74	50.77	55.89
Mean	52.78	48.86	55.82	49.93	47.21	53.21	49.20	55.02	54.65	46.47	51.31
AIA											
2019	0.24	0.52	0.37	-	0.31	-	-	0.40	-	0.58	0.40
2020	0.68	0.56	0.45	0.58	0.41	0.15	0.15	0.56	0.22	0.64	0.44
2021	0.21	0.62	0.21	0.30	0.24	0.06	0.09	0.19	0.15	0.20	0.23
Mean	0.38	0.57	0.34	0.44	0.32	0.11	0.12	0.38	0.18	0.47	0.36
Seeds											
Dry Matter											
2019	94.16	94.58	95.07	-	95.03	-	-	94.90	-	94.93	94.78
2020	93.13	92.82	92.65	93.85	92.94	92.81	92.66	92.57	92.72	92.74	92.89
2021	93.91	92.68	92.95	94.85	93.22	93.70	94.47	92.69	92.36	92.54	93.34
Mean	93.73	93.36	93.56	94.35	93.73	93.26	93.56	93.39	92.54	93.40	93.49
Ash											
2019	4.90	4.64	5.14	-	3.93	-	-	5.56	-	4.25	4.74
2020	5.07	4.81	4.55	4.68	4.36	4.29	4.93	4.79	3.82	4.16	4.55
2021	5.46	5.60	4.92	5.19	9.35	5.56	4.97	4.70	4.66	4.21	5.46
Mean	5.15	5.02	4.87	4.93	5.88	4.92	4.95	5.02	4.24	4.21	4.92
Crude Protein											
2019	22.63	21.00	22.80	-	23.55	-	-	19.20	-	21.43	21.77
2020	23.01	16.11	17.41	22.55	14.47	15.27	19.81	17.21	14.05	14.53	17.44
2021	25.18	16.75	19.01	24.01	17.63	21.02	21.84	16.05	17.87	14.73	19.41
Mean	23.61	17.95	19.74	23.28	18.55	18.15	20.82	17.48	15.96	16.89	19.24
Fat											
2019	18.15	21.88	27.84	-	28.70	-	-	22.85	-	21.55	23.50
2020	16.95	6.35	6.72	20.54	6.91	8.85	13.69	8.50	3.87	5.06	9.74
2021	17.89	4.83	8.38	25.75	5.60	3.44	16.95	5.51	3.24	3.71	9.53
Mean	17.66	11.02	14.31	23.15	13.74	6.14	15.32	12.29	3.55	10.11	12.73
Crude Fiber											
2019	33.92	33.88	25.53	-	28.73	-	-	30.62	-	33.53	31.04
2020	32.15	47.48	43.86	32.98	48.22	47.37	32.58	42.37	50.99	49.43	42.74
2021	28.18	41.70	37.66	25.45	42.07	36.18	31.94	44.55	43.42	47.99	37.91
Mean	31.42	41.02	35.68	29.21	39.67	41.77	32.26	39.18	47.20	43.65	38.11

continue

Stems											
Dry Matter											
2019	93.46	92.90	92.82	-	92.87	-	-	93.16	-	92.53	92.96
2020	93.56	94.09	93.77	93.98	93.87	93.75	93.28	93.61	94.06	93.61	93.76
2021	93.57	92.96	93.32	93.28	93.20	93.17	93.43	93.67	93.06	93.55	93.32
Mean	93.53	93.31	93.30	93.63	93.31	93.46	93.35	93.48	93.56	93.23	93.34
Ash											
2019	2.63	4.28	2.71	-	3.63	-	-	3.10	-	3.10	3.24
2020	2.46	3.95	2.79	2.90	3.17	3.71	2.98	3.00	2.85	3.42	3.12
2021	3.26	3.63	2.48	3.84	2.97	3.74	3.67	3.11	3.09	3.59	3.34
Mean	2.79	3.95	2.66	3.37	3.26	3.72	3.33	3.07	2.97	3.37	3.23
Crude protein											
2019	2.26	3.73	1.90	-	4.35	-	-	1.87	-	4.93	3.17
2020	2.97	4.90	3.78	3.55	4.30	4.96	3.80	4.85	5.32	5.27	4.37
2021	3.29	2.67	2.88	3.92	2.99	4.92	3.50	3.54	3.52	3.96	3.77
Mean	2.84	3.77	2.85	3.74	3.88	4.94	3.65	3.42	4.42	4.72	3.77
Fat											
2019	0.59	0.57	0.60	-	0.69	-	-	0.66	-	0.50	0.60
2020	0.78	0.86	1.01	0.57	1.00	0.69	1.20	0.78	0.58	0.54	0.80
2021	0.85	0.72	1.17	0.93	1.24	1.29	1.00	1.79	1.13	1.00	1.11
Mean	0.74	0.72	0.92	0.75	0.98	0.99	1.10	1.08	0.86	0.68	0.84
NDF											
2019	85.77	81.32	86.93	-	81.19	-	-	83.43	-	76.29	82.49
2020	83.03	82.07	79.82	84.88	79.58	76.94	82.49	82.17	78.57	78.97	80.85
2021	82.93	88.29	83.41	84.85	84.56	83.30	83.37	85.31	86.10	80.65	84.28
Mean	83.91	83.90	83.38	84.86	81.78	80.12	82.93	83.64	82.34	78.64	82.54
ADF											
2019	68.01	64.64	70.08	-	64.91	-	-	68.08	-	61.68	66.23
2020	63.82	63.30	60.77	64.97	63.43	60.03	65.02	63.17	62.02	61.82	62.84
2021	65.10	70.44	66.20	66.59	67.78	67.08	66.43	46.98	70.19	65.33	65.21
Mean	65.64	66.13	65.68	65.78	65.37	63.56	65.73	59.41	66.11	62.94	64.76
AIA											
2019	0.22	0.31	0.37	-	0.43	-	-	0.33	-	0.44	0.35
2020	0.13	0.36	0.45	0.63	0.24	0.59	0.51	0.24	0.53	0.69	0.44
2021	0.23	0.25	0.13	0.15	0.15	0.30	0.04	0.13	0.09	0.19	0.17

continue											
Mean	0.19	0.31	0.32	0.39	0.27	0.44	0.28	0.23	0.31	0.44	0.32
Leaves											
Dry Matter											
2019	91.01	90.30	90.95	-	90.71	-	-	90.30	-	90.45	90.62
2020	91.85	92.02	92.06	92.11	91.94	91.46	91.59	91.90	91.77	91.81	91.85
2021	91.65	90.56	91.75	91.64	90.72	92.33	91.94	91.57	90.58	91.82	91.45
Mean	91.50	90.96	91.59	91.87	91.12	91.90	91.76	91.25	91.18	91.36	91.31
Ash											
2019	12.79	21.70	20.75	-	17.92	-	-	18.71	-	20.81	18.78
2020	11.59	18.55	11.64	12.69	14.57	21.83	14.93	12.91	15.29	18.89	15.29
2021	16.72	20.75	12.25	17.50	15.48	12.40	16.91	15.47	15.94	15.18	15.86
Mean	13.70	20.33	14.88	15.10	15.99	17.12	15.92	15.70	15.61	18.29	16.64
Crude Protein											
2019	18.50	17.45	13.12	-	17.03	-	-	11.80	-	17.44	15.89
2020	20.92	23.39	21.30	19.77	21.34	18.10	19.02	20.35	21.10	21.78	20.71
2021	19.79	19.90	22.60	19.55	19.20	24.26	19.46	20.95	20.63	22.50	20.89
Mean	19.74	20.25	19.01	19.66	19.19	21.18	19.24	17.70	20.86	20.57	19.16
Fat											
2019	0.59	0.57	0.60	-	0.69	-	-	0.66	-	0.50	0.60
2020	0.78	0.86	1.01	0.57	1.00	0.69	1.20	0.78	0.58	0.54	0.80
2021	0.85	0.72	1.17	0.93	1.24	1.29	1.00	1.79	1.13	1.00	1.11
Mean	0.74	0.72	0.92	0.75	0.98	0.99	1.10	1.08	0.86	0.68	0.84
Crude Fiber											
2019	10.25	11.01	11.81	-	10.48	-	-	9.91	-	11.64	10.85
2020	11.83	10.79	11.88	13.21	11.04	10.65	9.31	11.26	9.88	12.35	11.22
2021	10.88	14.22	14.63	10.29	15.38	11.22	9.14	14.83	16.08	12.62	12.93
Mean	10.99	12.00	12.77	11.75	12.30	10.93	9.22	12.00	12.98	12.21	11.67
NDF											
2019	25.56	29.73	27.57	-	27.30	-	-	24.04	-	28.05	27.04
2020	27.53	24.30	27.23	28.02	27.47	23.71	24.24	25.88	25.45	27.15	26.10
2021	28.56	29.93	34.28	25.36	36.55	21.26	23.88	32.90	38.25	27.97	29.89
Mean	27.22	27.99	29.69	26.69	30.44	22.48	24.06	27.60	31.85	27.72	27.68
ADF											
2019	14.72	18.15	18.67	-	16.84	-	-	15.68	-	17.05	16.85
2020	16.48	14.70	15.16	16.85	14.29	13.27	14.79	13.66	14.22	15.65	14.91

continue											
2021	16.17	18.64	21.24	15.10	21.98	12.48	13.78	20.10	23.37	16.43	17.93
Mean	15.79	17.16	18.36	15.98	17.70	12.87	14.28	16.48	18.80	16.38	16.56
AIA											
2019	0.4	0.6	1.19	-	0.65	-	-	0.44	-	0.85	0.69
2020	0.22	0.28	0.15	0.50	0.30	0.47	0.97	0.09	0.30	0.24	0.35
2021	0.32	0.51	0.30	0.47	0.45	0.44	0.30	0.55	0.62	0.04	0.40
Mean	0.31	0.47	0.55	0.49	0.47	0.45	0.64	0.36	0.46	0.38	0.48
Cake											
Dry Matter											
2019	94.07	94.04	93.87	-	93.33	-	-	93.48	-	93.96	93.79
2020	94.40	94.51	94.68	95.26	94.91	94.99	94.58	94.46	94.64	94.52	94.70
2021	93.96	95.34	94.73	94.42	94.81	95.33	94.43	94.81	94.16	93.67	94.57
Mean	94.15	94.63	94.43	94.84	94.35	95.16	94.51	94.25	94.40	94.05	94.35
Ash											
2019	6.22	5.83	5.74		5.09			6.63		5.10	5.77
2020	5.61	5.11	4.93	4.93	4.62	4.70	5.29	5.24	4.33	4.59	4.93
2021	5.10	4.93	4.48	4.77	4.24	4.54	4.74	4.81	4.02	4.27	4.59
Mean	5.64	5.29	5.05	4.85	4.65	4.62	5.01	5.56	4.18	4.65	5.10
Crude Protein											
2019	31.10	33.73	33.83	-	31.36	-	-	29.64	-	28.44	31.35
2020	32.60	22.67	20.25	29.59	17.11	22.66	27.41	22.40	13.75	16.94	22.54
2021	31.33	21.89	20.14	29.23	15.78	16.91	14.41	21.46	28.15	15.10	21.44
Mean	31.68	26.10	24.74	29.41	21.42	19.79	20.91	24.50	20.95	20.16	25.11
Fat											
2019	16.65	10.03	10.38	-	10.47	-	-	11.46	-	15.92	12.48
2020	16.25	13.76	11.59	20.06	15.18	15.81	21.59	12.29	6.91	9.54	14.30
2021	23.70	12.56	11.70	26.62	10.66	10.04	25.68	12.79	6.03	6.38	14.62
Mean	18.87	12.12	11.22	23.34	12.10	12.92	23.64	12.18	6.47	10.61	13.80
Crude Fiber											
2019	24.97	30.54	29.59	-	29.74	-	-	29.13	-	28.36	28.72
2020	26.97	37.41	38.04	26.75	39.64	36.52	26.93	36.28	41.91	43.32	35.38
2021	21.93	36.11	34.36	22.55	40.76	39.08	23.10	33.34	44.52	42.44	33.82
Mean	24.62	34.69	34.00	24.65	36.71	37.80	25.02	32.92	43.22	38.04	32.64

2.4.3 Chemical composition of hemp seed and cake

The average fat content (12.73%) of hemp seed from different varieties was lower than the reported values (30%) in the literature (Callaway 2004; Gibb et al. 2005; Silversides et al. 2005; Hessle et al. 2008; Karlsson et al. 2010; Vonapartis et al. 2015; Mierlita 2016; Wang et al. 2017; Habenau et al. 2018; Mierlita 2018). This variation in fat content of hempseed might be attributed to the agro-climatic conditions of the different years. The fat content of hemp seed was the highest in Eletta Campana variety and increased from 2020 to 2021 (from 20.54 to 25.75% on DM resp.).

Analysis of the oilseed residue of hempseed, called hemp cake revealed a high protein content (%DM), ranging from 13.75 to 33.83, whereas the fiber, ash, and moisture contents (%DM) were in the range of 21.93-44.52, 4.02–6.63, and 93.33-95.33 respectively. On average, the protein content (25.11% on DM) of the ten hemp varieties was less than the one reported in the literature for hempseed cake obtained by cold pressing (34.3 ± 2.1% on DM). The crude protein content of hemp cake was highest in CS variety and remained stable along the three years of cultivation (31.7 ± 0.8% on DM). The other varieties had good protein content in 2019 but it decreased dramatically in the two following years. The protein content of the hempseed cake was comparable to those reported in the literature proving that it can be used as an optimal protein source in animal feeding (Callaway 2004; Hessle et al. 2008; Karlsson et al. 2010; Mierlita 2018).

The method of extraction is very important not only to obtain a good quality oil but also to have a good oil yield. As other oil seeds, cold mechanical extraction of seed produces a cake that is higher in oil and so in fat content. The lipid content obtained on average (13.8% on DM) was a bit higher than the one reported in the literature (12.7 ± 2.8% on DM). The fat content of hemp cake was the highest in Fibranova variety and increased from 2020 to 2021 (from 21.59 to 25.68% on DM resp.).

2.4.4 Fatty acid composition of hemp seeds, oil and cake

The quality of the oil obtained by mechanical extraction is better than the one obtained by chemical extraction. As expected, the FA profiles of hemp oil and cake were very similar and similar to previous studies (Callaway 2004; Stambouli et al. 2005; Rovellini et al. 2013; Cozma et al. 2015; Mourot et al. 2015; Mierlita 2016; Mierlita 2018; Juodka et al. 2018). Generally, the studied hemp seeds and products were characterized by a high content of PUFA and a low MUFA and SFA. Regardless the variety and year of cultivation, the main FA in hemp seeds, oil and cake was linoleic acid showing average contents of 54.83, 54.93, and 55.46% respectively. The principal SFA in hemp seeds and products was palmitic. It is noteworthy that, of all the hempseed varieties evaluated, Eletta Campana showed the highest content of linolenic and linoleic acids. The total SFA, MUFA and PUFA of oil were resp. 12.59, 16.06 and 71.35% of total FA. Meanwhile the total SFA, MUFA and PUFA of cake were resp. 13.57, 14.58 and 71.84% of total FA. The palmitic (C16:0) acid represent the higher values of SFA in hemp seeds, oil and cake. For hemp cake and oil, the variety with the highest content of SFA was Santhica 27. The average values of the percentage of MUFA were similar for hemp seed and cake (14.96 and 14.58% of total FA resp.). For both hempseed products, cake and oil, the variety Carmaleonte was the highest in MUFA content (18.13 and 20.03% of total FA resp.). On average, for both hempseed products, the oleic acid (C18:1) is the most representative FA of this group (13.67 and 14.34% of total FA for cake and oil resp). The sum of PUFA were close for both hemp oil and cake (71.35 and 71.84% of total FA resp.). With respect of PUFA, an average of 70.19, 71.35 and 71.84 was found for seeds, oil and cake respectively. Eletta Campana variety showed the highest value of PUFA for seeds, oil and cake. Among PUFA, the linoleic acid (C18:2 n-6) and alpha-linolenic acid (C18:3 n-3) are the most representatives. Along the three years of cultivation, the values of SFA, MUFA and PUFA remained stable.

Table 4. Fatty acid profile of hemp seed and co-products of ten hemp varieties along three consecutive years

	CS	Carmaleonte	Codimono	E.Campana	Felina32	Ferimon	Fibranova	Futura75	Santhica27	USO31
Seeds										
Total SFA										
2019	13.37	12.47	12.95	-	11.66	-	-	14.90	-	-
2020	11.61	15.84	15.98	12.05	16.01	14.95	12.83	15.40	17.97	15.92
2021	11.34	16.24	15.40	12.17	16.31	18.93	13.00	16.36	18.90	18.53
Total UFA										
2019	86.63	87.53	87.05	-	88.34	-	-	85.10	-	-
2020	88.39	84.17	84.02	87.95	83.99	85.05	87.17	84.60	82.03	84.08
2021	88.66	83.76	84.60	87.83	83.69	81.07	87.00	83.64	81.10	81.47
Total MUFA										
2019	14.86	22.81	14.03	-	15.18	-	-	17.81	-	-
2020	11.63	17.26	16.39	11.41	15.24	13.50	12.86	15.18	12.91	17.00
2021	14.55	20.90	13.36	12.15	14.44	15.33	11.66	14.49	14.78	14.38
Total PUFA										
2019	71.77	64.72	73.02	-	73.15	-	-	67.29	-	-
2020	76.77	66.90	67.63	76.54	68.74	71.56	74.31	69.42	69.12	67.08
2021	74.11	62.86	71.24	75.68	69.26	65.75	75.33	69.15	66.32	67.09
Total n-6										
2019	57.51	56.05	56.02	-	58.80	-	-	56.71	-	-
2020	56.40	54.05	53.17	57.37	56.11	57.97	57.48	55.71	53.95	55.10
2021	64.48	52.10	54.50	57.50	55.15	53.30	57.42	55.42	52.93	53.52
Total n-3										
2019	14.26	8.67	17.00	-	14.35	-	-	10.57	-	-
2020	19.52	11.74	13.48	18.40	11.62	12.59	15.87	12.74	14.03	10.78
2021	9.54	10.73	16.71	18.13	14.07	12.41	17.87	13.69	13.35	13.52

continue n-6/n-3										
2019	4.03	6.47	3.30	-	4.10	-	-	5.36	-	-
2020	2.89	4.61	3.94	3.12	4.83	4.61	3.62	4.37	3.85	5.11
2021	6.76	4.86	3.26	3.17	3.92	4.30	3.21	4.05	3.97	3.96
Oil										
Total SFA										
2019	14.54	10.67	9.30	-	13.57	-	-	16.35	-	10.99
2020	11.68	12.36	12.46	10.99	21.51	13.03	11.35	12.65	-	13.45
2021	10.99	12.54	12.27	10.28		13.22	10.67	12.08	-	
Total UFA										
2019	85.46	89.33	90.70	-	86.43	-	-	83.64	-	89.01
2020	88.32	87.64	87.54	89.01	78.50	86.97	88.65	87.35	-	86.55
2021	89.01	87.46	87.73	89.72	-	86.78	89.33	87.92	-	
Total MUFA										
2019	14.86	20.92	16.98	-	14.103			17.16	-	17.6022
2020	12.80	18.53	15.98	11.39	21.61	15.98	13.16	15.76	-	15.52
2021	13.64	20.65	16.04	12.87		17.14	13.88	16.72	-	
Total PUFA										
2019	70.61	68.41	73.71	-	72.33	-	-	66.48	-	71.40
2020	75.52	69.10	71.56	77.62	56.89	70.99	75.49	71.60	-	71.04
2021	75.38	66.82	71.69	76.85	-	69.65	75.45	71.20	-	
Total n-6										
2019	53.13	54.42	53.31	-	57.97	-	-	52.34	-	56.77
2020	57.32	57.10	55.94	58.04	49.23	57.81	58.61	57.59	-	56.89
2021	57.47	55.25	55.02	57.66	-	57.76	58.89	56.77	-	
Total n-3										
2019	17.48	13.99	20.40	-	14.36	-	-	14.13	-	14.63

continue										
2020	17.54	11.11	14.76	18.95	6.20	12.38	16.20	13.19	-	13.14
2021	17.91	11.57	16.67	19.19	-	11.88	16.57	14.43	-	-
n-6/n-3										
2019	3.04	3.89	2.61	-	4.04	-	-	3.70	-	3.88
2020	3.27	5.14	3.79	3.06	7.94	4.67	3.62	4.37	-	4.33
2021	3.21	4.78	3.30	3.00	-	4.9	3.55	3.93	-	-
Cake										
Total SFA										
2019	11.90	12.99	11.47	-	12.40	-	-	12.35	-	12.02
2020	13.44	14.60	14.18	12.79	14.58	12.32	11.77	13.49	21.73	-
2021	12.44	13.44	13.00	11.66	16.11	13.15	11.16	14.10	15.27	17.01
Total UFA										
2019	88.10	87.01	88.53	-	87.60	-	-	87.65	-	87.98
2020	86.57	85.40	85.83	87.21	85.42	87.68	88.23	86.51	78.28	
2021	87.56	86.56	87.00	88.34	83.89	86.85	88.84	85.90	84.73	82.99
Total MUFA										
2019	12.90	16.40	14.12	-	13.38	-	-	14.05	-	16.30
2020	11.72	18.87	15.61	11.04	14.77	13.65	12.82	15.68	16.76	-
2021	12.12	19.12	11.66	11.91	14.55	16.51	13.69	15.43	15.55	15.94
Total PUFA										
2019	75.20	70.61	74.41	-	74.22	-	-	73.60	-	71.68
2020	74.85	66.54	70.22	76.17	70.65	74.03	75.41	70.83	61.51	-
2021	75.44	67.44	75.33	76.43	69.33	70.34	75.15	70.47	69.18	67.05
Total n-6										
2019	58.37	58.58	57.30	-	59.92	-	-	59.53	-	58.83
2020	56.65	55.09	54.85	57.03	57.35	61.14	58.14	56.82	49.86	-
2021	56.16	55.08	57.42	56.63	55.73	57.65	58.34	55.45	56.77	53.78

continue

Total n-3										
2019	17.08	12.03	17.11	-	14.31	-	-	14.35	-	12.85
2020	17.48	10.46	14.57	18.49	12.32	12.11	16.51	13.14	10.28	-
2021	19.24	12.31	17.87	19.76	13.57	12.62	16.81	14.98	12.30	13.23
n-6/n-3										
2019	3.42	4.87	3.35	-	4.19	-	-	4.15	-	4.58
2020	3.24	5.27	3.76	3.08	4.65	5.05	3.52	4.32	4.85	
2021	2.92	4.47	3.21	2.87	4.11	4.57	3.47	3.70	4.61	4.06

2.5 Conclusions

A comprehensive composition study on ten varieties of hemp as a whole plant, its botanical fractions and products (oil and cake) along three years of cultivation in Northern Italy was presented. Despite the high proportion of stems that increased dramatically along the years of study, the nutritional composition of hemp supports its potential as a highly beneficial food or feed. The ten varieties evaluated contained interesting amounts of fat and protein, and hemp seed cake showed the highest contents of these two nutrients. Additionally, seeds, leaves and cake could be used as a good source of protein. Moreover, the fatty acid profile of hemp oil and cake are very interesting, considering the high level of PUFA and the good ratio between n-6 and n-3 fatty acids. Some differences on fatty acid profile were observed among varieties whereas the effect of the year was meaningless. CS and Eletta campana were the most promising varieties because of their protein and fat content respectively, the last one also showed the highest levels of poly unsaturated fatty acids. Future studies that keep evaluating the nutritional values of different hemp varieties are needed in order to design and develop high quality products for consumers and help breeders to improve agronomic aspects based on the nutrition values.

2.6 References

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Chapter 3.

Chemical Characterization of 29 Industrial Hempseed (*Cannabis sativa L.*) Varieties

3.1 Abstract

Hemp is considered one of the potential novel crops for human and animal nutrition. This study aimed to determine the complete chemical composition of 29 different varieties of whole hempseeds. Fatty acid composition, amino acid profile, mineral composition and cannabinoids content were also evaluated. All hempseed varieties were milled to obtain whole hempseed flour. Differences between hempseed varieties were significant ($P<0.05$) for all measured parameters. Proximate composition reported crude protein and fat contents varied from 21.6-28.9% and 21.1-35.7%, respectively. Fatty acid profiles revealed that the three major fatty acids were linoleic acid, 52.79-57.13% followed by α-linolenic acid, 12.62–20.24%, and oleic acid, 11.08-17.81%. All essential amino acids were detected in all varieties, with arginine (12.66-17.56 mg/100g protein) present in abundance. Lysine was limiting. Substantial differences were found in the mineral content, potassium (509.96 -1182.65 mg/100g) and iron (5.06-32.37 mg/100mg) were the main macro and microminerals found. All cannabinoids were found in small traces and tetrahydrocannabidiol (THC) was only detected in 5 varieties. To conclude, the nutritional composition of hempseeds with hull makes them suitable to be added into humans or animals' diet as a highly beneficial novel ingredient.

3.2 Introduction

Industrial hemp (*Cannabis sativa L.*) seeds have a beneficial nutritional composition, as they are a rich source of protein, unsaturated fatty acids, and some minerals [1]. Even though studies on the chemical composition and nutrient contents of hempseed show significant variation among hemp varieties, which is known can be directly correlated with the genotypes and the environment (rainfall, temperature, soil, etc.) some data is available. Hempseed contains about 25% of protein. A total of 181 proteins have been identified in hempseeds with the main ones being the globular-type albumin (25-37%) and the legumin-type globulin edestin (67-75%) [1]. Hempseed is an excellent source of digestible amino acids as it contains high levels of arginine, aspartic acid and glutamic acid [2]. Concerning fatty acids, the polyunsaturated ones are predominant in hempseeds. It contains significant amounts of linoleic acid, which accounts for more than half of total fatty acids. The remaining fatty acid content is comprised of α-linolenic acid (16–19%), oleic acid (12–17%), palmitic acid (5–8%), γ-linolenic acid (1–3%), and some other minor fatty acids [3,4]. The total mineral content of hempseed is often reflected by the ash content [1] and the main minerals are calcium, magnesium, potassium, iron, manganese, copper and zinc. Some differences between the mineral content have been reported due to environmental conditions, agricultural practices and varieties.

Cannabinoids are among the 400 different chemical substances that have been isolated from hemp. They are mostly in the inflorescence of the plant and the most abundant are cannabidiol (CBD) and tetrahydrocannabidiol (THC). CBD is the main non-psycotrophyc cannabinoid, while THC is the only psychoactive component in hemp. Even though, industrial hemp has less than 0.2% of THC [5], this is a critical consideration for both consumers and industries because of the potential accumulation of THC in animal tissues and its effect on animal health, production, and food product quality [8,9]. In fact, it has been proved that cannabinoids of hemp by-products can transfer into milk when they are included in dairy cows' diet [6,7]. Therefore, minimizing the risk of psychoactive effects associated with THC to make industrial hemp seeds safe for consumption needs to be guaranteed by always measuring its content. In this way, consumers and industries can relay and confidently incorporate these seeds into various products, such as food items and other nutritional supplements, knowing that they offer valuable nutrients without the concerns associated with high THC levels.

Due to its impressive nutritional profile and bioactive compounds already mentioned, hemp seeds are often considered nutraceuticals because they provide health bene-fits beyond basic nutrition. The remarkable potential of hempseed as an innovative candidate for both food and feed applications underscores the impetus behind the cultivation of diverse industrial hemp varieties in various countries. The aim of this study was to determine a complete chemical composition and nutritional characteristics of 29 different hempseed varieties, and also contrast it with soybean.

3.3 Materials and Methods

3.3.1 Materials

Whole hempseeds of 29 varieties were used in this study (Table 1), some of them monoecious and some of them dioecious. They are originally from 8 different countries and 7 of them are not registered in the European Union so they are not included in the Plant Variety Database of the European Commission [10]. The place of cultivation was the Institute of Field and Vegetables Crops, Department for Alternative Crops, Bački Petrovac, Serbia (45.336500°N 19.671355°E). The soil was alluvial chernozem with a pH of 7.2 and the previous crop was sorghum. The soil preparation consisted in deep plowing, followed by disking and cultivation for the establishment of a suitable seedbed. Before plowing in fall, a fertilization with nitrogen, phosphorus and potassium was done in a proportion of 16:16:16 at 300 kg/ha. The sowing was on April, 2021. Each variety had 3 rows of 10 m long and the distance between plants was 50 cm. Plots were kept weed-free by mechanical cultivation until the fifth week after the emergency. The harvest was done manually in October, 2021.

Soybean rubin cv. was cultivated at the Institute of Field and Vegetables Crops, Department of Legumes, Rimski šančevi, Serbia (45.329146°N 19.835969°E) in 2022. The soil was chernozem with homogeneous texture and a well-aggregated structure.

Some climatic parameters like temperature and precipitation (Figure 1) of the hemp cultivation site were measured at the meteorological station located approximately 500 meters away from the experiment.

Table 1. Industrial hempseed varieties analyzed and their main characteristics.

Variety	Origin	Sex	EU Registration
Antal	Hungary	Dioecious	No
Bacalmas	Hungary	Dioecious	No
Carmagnola	Italy	Dioecious	Yes
Chameleon	Holland	Monoecious	Yes
Dioica 88	France	Dioecious	Yes
Epsilon 88	France	Monoecious	Yes
Fedora 17	France	Monoecious	Yes
Felina 32	France	Monoecious	Yes
Ferimon FR 8194	France	Monoecious	Yes
Fibrol	Hungary	Monoecious	Yes
Futura 75	France	Monoecious	Yes
Helena	Serbia	Monoecious	Yes
KC Dora	Hungary	Monoecious	Yes
KC Virtus	Hungary	Dioecious	Yes
KC Zuzana	Hungary	Monoecious	Yes
Kina	China	Dioecious	No
Kompolti	Hungary	Dioecious	Yes
Lovrin110	Romania	Dioecious	Yes
Marina	Serbia	Dioecious	Yes
Monoica	Hungary	Monoecious	Yes
Novosadska	Serbia	Dioecious	No
Novosadska+	Serbia	Dioecious	No
Santhica 23	France	Monoecious	Yes
Secuieni jubileu	Romania	Monoecious	Yes
Silesia	Poland	Monoecious	No
Simba	Serbia	Dioecious	No
Tiborszallasi	Hungary	Dioecious	Yes
Tisza	Hungary	Dioecious	Yes
Wojko	Poland	Monoecious	Yes

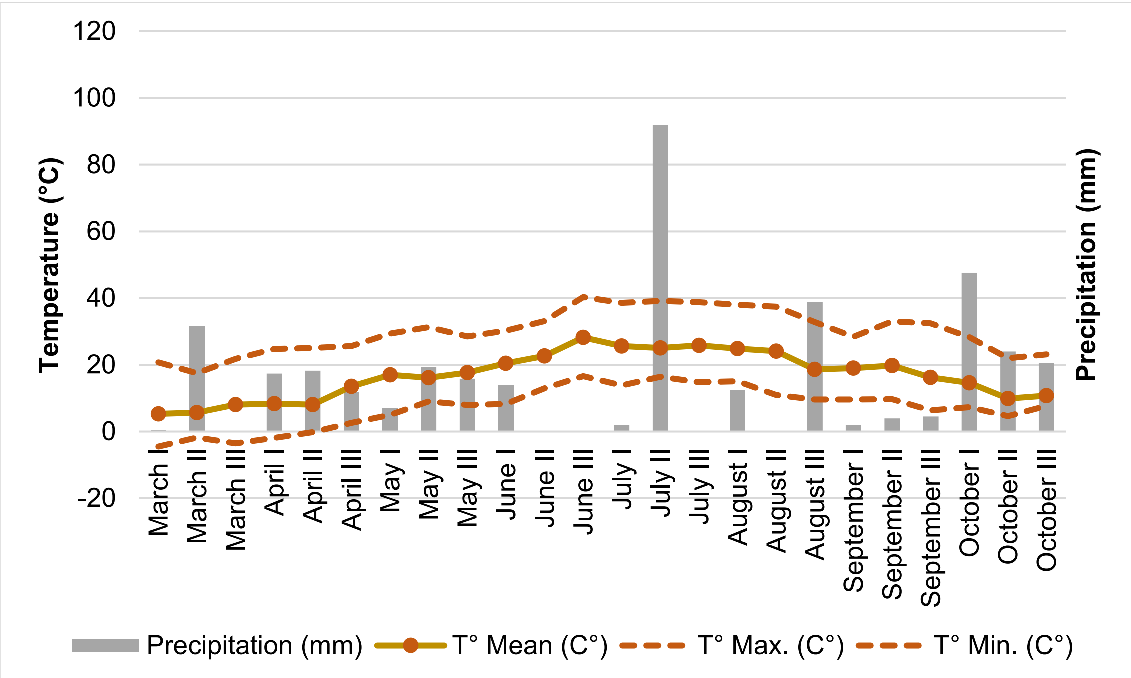

Figure 1. Climate conditions (precipitation and temperature) at the hemp field.

3.3.2 Sample preparation

Industrial hempseeds and soybean with hulls were milled (FOSS KN295 Knifetec, Labtec Line, Hillerod, Denmark) to obtain flour at the Institute of Food Technology (FINS) of the University of Novi Sad, Serbia. All analyses were performed using 2 replicates.

3.3.3 Chemical composition

Moisture, ash, crude protein (CP) (ISO20483) [11] and lipids (EE) were determined according to the AOAC international standards [12] at the Institute of Food Technology (FINS) of the University of Novi Sad, Serbia. Total carbohydrates (CHO) were calculated by difference in percentages: 100% - (moisture + ash + crude protein + lipids) %.

3.3.4 Fatty acid profile

Preparation of methyl esters of fatty acids and determination by capillary gas chromatography (Agilent 7890A system, Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and a SP-2560 fused silica capillary column (100 m x 0.25 mm x 0.20 µm film thickness) were done following official procedures (ISO12966-2, ISO12966-4) [13,14] at the Institute of Food Technology (FINS) of the University of Novi Sad, Serbia. A methyl ester standard mix of 37 fatty acids, Supelco 37 FAME mix (Supelco, Bellefonte, USA) was used as an internal standard for the analysis of each sample. Results were expressed as percentages of total FA.

3.3.5 Amino acid profile

The amino acid composition was determined by ion exchange chromatography using an automatic amino acid analyzer Biochrom 30+ (Biochrom, Cambridge, UK) at the Institute of Food Technology (FINS) of the University of Novi Sad, Serbia following the method described by Spackman et al. (1958) [15]. The technique was based on amino acid separation using strong cation exchange chromatography, followed by the ninhydrin colour reaction and photometric detection at 570 nm, except for proline, which was detected at 440 nm. L-norleucine (Sigma-Aldrich, St. Louis, USA) was used as an internal standard in each sample analysis.

Samples were previously hydrolysed in 6M HCl (Merck, Germany) at 110 °C for 24 h. While alkaline hydrolysis with 4M NaOH was used for the determination of tryptophan. After hydrolysis, samples were cooled at room temperature and dissolved into 25 mL of loading buffer (pH 2.2) (Biochrom, Cambridge, UK). Subsequently, filtered through 0.22 µm pore size PTFE filter (Plano, Texas, USA) and transferred into a vial (Agilent Technologies, USA) and stored in a refrigerator prior to analysis.

The amino acid peaks were identified by comparison of retention times with the standard purchased from Sigma Aldrich (Amino Acid Standard Solution, Sigma-Aldrich, St. Louis, USA). The results were expressed as total protein basis (g/100 g protein) or whole seed basis (g/100 g seeds).

3.3.6 Mineral composition

The mineral composition (Ca, Mg, K, Fe, Mn, Cu, Zn, Na) was determined by flame atomic absorption spectroscopy (AAS) at the Institute of Food Technology (FINS) of the University of Novi Sad, Serbia according to the official method ISO6869 [16]. This methodology did not allow to determine the content of phosphorus.

3.3.7 Cannabinoid analysis

The cannabinoid analysis was done at the Institute of Field and Vegetable Crops of the University of Novi Sad, Serbia according to the procedure described by Zeremski *et al*. (2018) [17]. Absolute ethanol (10 ml) was added to 2 g of dried homogenized sample in an erlenmeyer with a stopper. The solution was sonicated for 15 min and then centrifuged at 10 000 rpm for 5 min. The supernatant was transferred into a GC vial. The decarboxylation step of acidic form of CBD and THC was achieved in the GC-MS inlet at a temperature of 280 °C.

Analysis of cannabinoids was performed on Agilent 6890N gas chromatograph equipped with mass spectrum detector. The separation was performed on a fused silica capillary column (HP-5, 30 m x 0.25 mm i.d., and 0.25 µm film thickness). Helium was used as carrier gas at a constant flow of 1 ml/min. The temperature program was as follows: initial temperature of 200 °C was held for 2 min, then increased to 240 °C at a rate of 10 °C/min, and kept for 10 min. The injector and detector temperatures were set at 280 and 230 °C, respectively. The injected sample volume was 1.5 µl and split ratio was 1:20. Individual analytical standards for cannabidiol (CBD) and cannabinol (CBN) were used for calibration. Quantification of THC was performed with CBN analytical standard in accordance to the method given by Poortman-van der Meer and Huizer (1999) [18] and expressed as % in dry weight.

3.3.8 Statistical analysis

All analyses were performed in duplicates. The results were subjected to analysis of variance (ANOVA) by using a completely randomized design method (SAS Inst. Inc., Cary, NC, USA). Pair-wise comparisons were done using the Tukey test. Significant levels were considered at $P < 0.05$.

3.4 Results

3.4.1. Chemical composition

Proximate analyses of 29 hempseed varieties are shown in Table 2. Some differences in dry matter (DM), CP, EE, CHO and ash contents were found ($P<0.05$). Dry matter among all hempseeds ranged from 90.34 to 93.52%. The lowest value was recorded from Marina v. and the highest from Ferimon FR 8194 v. The content of crude protein of hempseeds ranged from 21.63 to 28.92% DM. The lowest value was recorded from Monoica v. and the highest from Tisza v. Fat content of hempseeds ranged from 21.12 to 35.67% DM. The lowest value was recorded from Epsilon 88 v. and the highest from Fibrol v. Total carbohydrates content from hempseeds ranged from 25.49 to 43.00% DM. The lowest value was recorded from Tisza v. and the highest from Epsilon 88 v. Ash content from hempseeds ranged from 4.40 to 7.49% DM. The lowest value was recorded from Wojko v. and the highest from KC Virtus v. Tisza v. was the most balanced for crude protein and fat content. In comparison to the sample of soybean, the crude protein content in this seed is much higher (41.79 VS 25.17%) whereas the fat content (18.65 VS 31.72%) is much lower than the average of all hempseeds. Similar values of total carbohydrates content were found between soybean and the mean of all hempseed varieties.

Table 2. Nutritional composition (%DM) of 29 hempseed varieties.

Variety	DM	CP	EE	CHO	Ash
Antal	92.87 ± 0.05^{kl}	23.04 ± 0.01^{no}	$32.09 \pm 0.05^{g-i}$	$31.26 \pm 0.05^{e-g}$	6.47 ± 0.07^{b}
Bacalmas	92.63 ± 0.16^{mn}	$25.38 \pm 0.27^{e-h}$	$28.91 \pm 0.13^{o-q}$	32.25 ± 0.56^{de}	6.08 ± 0.01^{cd}
Carmagnola	93.36 ± 0.06^{bc}	26.73 ± 0.13^{cd}	$33.61 \pm 0.15^{c-e}$	$27.84 \pm 0.28^{j-l}$	$5.18 \pm 0.06^{g-i}$
Chameleon	$93.10 \pm 0.02^{f-h}$	25.67 ± 0.02^{ef}	$31.83 \pm 0.01^{h-j}$	30.71 ± 0.05^{gh}	4.88 ± 0.04^{jk}
Dioica 88	$93.29 \pm 0.05^{b-d}$	25.07 ± 0.17^{gh}	$29.85 \pm 0.11^{m-o}$	33.38 ± 0.27^{cd}	4.97 ± 0.04^{ij}
Epsilon 88	92.36 ± 0.07^{o}	22.56 ± 0.03^{o}	21.12 ± 0.14^{r}	43.00 ± 0.04^{a}	5.67 ± 0.07^{e}
Fedora 17	92.54 ± 0.04^{n}	26.34 ± 0.17^{d}	$30.63 \pm 0.12^{k-m}$	$30.96 \pm 0.05^{e-h}$	4.60 ± 0.04^{ml}
Felina 32	$92.94 \pm 0.06^{i-k}$	$25.13 \pm 0.02^{f-h}$	$32.45 \pm 0.09^{f-h}$	30.31 ± 0.01^{gh}	$5.05 \pm 0.05^{h-j}$
Ferimon FR8194	93.52 ± 0.05^{a}	26.54 ± 0.04^{d}	34.97 ± 0.27^{ab}	26.47 ± 0.37^{mn}	5.53 ± 0.01^{ef}
Fibrol	$93.06 \pm 0.09^{g-i}$	$25.46 \pm 0.09^{e-g}$	35.67 ± 0.05^{a}	26.38 ± 0.13^{mn}	5.54 ± 0.01^{fe}
Futura 75	93.40 ± 0.01^{ab}	26.36 ± 0.32^{d}	34.16 ± 0.07^{bc}	$26.72 \pm 0.01^{l-n}$	6.16 ± 0.25^{c}
Helena	90.76 ± 0.01^{p}	$25.55 \pm 1.28^{e-g}$	$29.36 \pm 0.29^{n-p}$	29.62 ± 1.74^{hi}	6.24 ± 0.19^{c}
KC Dora	$93.17 \pm 0.01^{d-g}$	$24.84 \pm 0.09^{h-j}$	$33.15 \pm 0.07^{d-f}$	28.94 ± 0.14^{ij}	6.24 ± 0.02^{c}
KC Virtus	92.76 ± 0.07^{lm}	$23.33 \pm 0.18^{l-n}$	$33.04 \pm 0.07^{e-g}$	28.90 ± 0.10^{ij}	7.49 ± 0.06^{a}
KC Zuzana	$93.13 \pm 0.02^{e-h}$	23.23 ± 0.18^{mn}	$31.55 \pm 0.13^{h-k}$	32.70 ± 0.32^{d}	5.64 ± 0.01^{e}
Kina	$93.24 \pm 0.07^{c-e}$	$25.00 \pm 0.25^{g-i}$	34.24 ± 0.25^{bc}	28.60 ± 0.59^{ij}	5.40 ± 0.03^{fg}
Kompolti	92.91 ± 0.02^{jk}	27.15 ± 0.23^{c}	$33.44 \pm 0.02^{c-f}$	26.08 ± 0.65^{mn}	6.23 ± 0.37^{c}
Lovrin110	$93.00 \pm 0.06^{h-k}$	26.85 ± 0.01^{cd}	$31.06 \pm 0.06^{j-l}$	29.84 ± 0.06^{hi}	5.24 ± 0.06^{gh}
Marina	90.34 ± 0.01^{q}	23.79 ± 0.48^{cd}	$31.91 \pm 0.67^{h-j}$	$28.51 \pm 1.31^{i-k}$	6.13 ± 0.14^{cd}
Monoica	$92.93 \pm 0.06^{i-k}$	21.63 ± 0.14^{p}	$31.69 \pm 0.03^{h-j}$	34.25 ± 0.09^{c}	5.36 ± 0.03^{fg}
Novosadska	$93.00 \pm 0.05^{h-k}$	23.89 ± 0.07^{kl}	28.12 ± 2.43^{q}	36.26 ± 2.41^{b}	4.73 ± 0.01^{kl}
Novosadska+	$93.04 \pm 0.06^{g-j}$	$24.47 \pm 0.13^{i-k}$	$30.18 \pm 0.01^{l-n}$	33.10 ± 0.01^{cd}	5.29 ± 0.07^{g}
Santhica 23	$93.20 \pm 0.14^{e-d}$	27.86 ± 0.10^{b}	$31.85 \pm 0.20^{h-j}$	28.20 ± 0.33^{jk}	5.29 ± 0.11^{g}
Secuieni jubileu	$92.93 \pm 0.07^{i-k}$	$25.38 \pm 0.01^{e-h}$	$32.03 \pm 0.10^{h-j}$	$30.80 \pm 0.03^{f-h}$	4.72 ± 0.06^{kl}
Silesia	$92.92 \pm 0.04^{i-k}$	24.38 ± 0.03^{jk}	$31.44 \pm 0.23^{i-k}$	$32.15 \pm 0.39^{d-f}$	4.96 ± 0.09^{ij}
Simba	92.59 ± 0.05^{n}	$25.15 \pm 0.02^{f-h}$	35.01 ± 0.25^{ab}	25.87 ± 0.28^{mn}	6.56 ± 0.01^{b}
Tiborszalassi	$92.99 \pm 0.08^{h-k}$	25.75 ± 0.03^{e}	$34.13 \pm 0.04^{b-d}$	$27.20 \pm 0.17^{k-m}$	5.91 ± 0.19^{d}
Tisza	92.87 ± 0.01^{kl}	28.92 ± 0.05^{a}	$33.91 \pm 0.36^{c-e}$	25.49 ± 0.27^{n}	4.55 ± 0.03^{lm}
Wojko	$93.27 \pm 0.16^{b-e}$	$24.47 \pm 0.18^{i-k}$	28.48 ± 0.19^{pq}	35.92 ± 0.66^{b}	4.40 ± 0.14^{m}
Mean ± SD	92.83±0.68	25.17±1.61	31.72±2.85	30.40±3.82	5.54±0.72
SEM	0.0699	0.2877	0.4917	0.6687	0.1124
Soybean	89.91±0.01	41.79±0.18	18.65±0.17	34.36±0.50	5.20±0.16

DM: dry matter; CP: crude protein; EE: ether extract; CHO: total carbohydrates. Means with different superscripts are significantly different at $P < 0.05$.

3.4.2 Fatty acid composition

The FA content (% of FAME) of all the hempseed varieties are presented in Table 3 and Table 4. The different varieties of hempseeds showed statistically significant differences for all individual FA and the fatty acid groups ($P<0.05$). The main FA of hempseeds were linoleic acid

(18:2n-6), from 52.79 to 57.13; α-linolenic acid (C18:3 n-3), from 12.62 to 20.24 and oleic acid (C18:1), from 11.08 to 17.81.

Hempseeds are characterized by a high proportion of unsaturated fatty acids, from which PUFA represented a high proportion ranged from 70.73 to 71.27. In terms of SFA, it ranged from 10.02 to 13.01. Palmitic acid (C16:0) is the leading FA of this group with a concentration that ranged from 3.79 to 8.13. Kina and Santhica 23 v. were the highest in PUFA and lowest in SFA. The ω6/ω3 ratio ranged from 2.86 to 4.71. The lowest value corresponding to the varieties Dioica 88 and Kina, whereas the highest to Wojko.

Table 4. Fatty acid profile (% of FAME) of 29 hempseed varieties.

	Σ SFA	**Σ MUFA**	**Σ PUFA**	**ω6/ω3**
Antal	11.77±0.04^{gh}	15.71±0.05^{hi}	72.52±0.01^{p}	3.83±0.01^{l}
Bacalmas	12.22±0.01^{f}	16.21±0.01^{f}	71.57±0.01^{tu}	3.98±0.01^{h}
Carmagnola	10.89±0.02^{k}	14.23±0.02^{m}	74.88±0.01^{j}	3.27±0.01st
Chameleon	12.37±0.01^{de}	14.41±0.01^{l}	73.22±0.01^{m}	4.47±0.01^{c}
Dioica 88	10.07±0.07^{o}	11.99±0.07^{u}	77.94±0.01^{b}	2.86±0.01^{x}
Epsilon 88	12.95±0.08^{a}	15.78±0.08^{h}	71.27±0.01^{v}	4.35±0.01^{d}
Fedora 17	12.32±0.02^{ef}	15.96±0.02^{g}	71.72±0.01^{s}	4.68±0.01^{b}
Felina 32	11.17±0.02^{j}	14.06±0.01^{o}	74.77±0.02^{k}	3.87±0.01^{j}
Ferimon FR 8194	10.84±0.01^{k}	11.86±0.01^{v}	77.30±0.01^{d}	3.92±0.01^{i}
Fibrol	11.82±0.07^{g}	16.93±0.07^{c}	71.25±0.01^{v}	4.24±0.01^{e}
Futura 75	10.78±0.21^{kl}	12.28±0.01^{t}	76.94±0.21^{e}	3.33±0.01^{r}
Helena	10.70±0.01^{lm}	11.80±0.01^{v}	77.50±0.01^{c}	3.04±0.01^{v}
KC Dora	10.83±0.01^{k}	12.93±0.01^{s}	76.24±0.01^{g}	3.26±0.01^{t}
KC Virtus	11.28±0.07^{ij}	12.00±0.07^{u}	76.72±0.01^{f}	3.28±0.01^{s}
KC Zuzana	11.34±0.01^{i}	17.93±0.01^{a}	70.73±0.01^{w}	3.56±0.01^{o}
Kina	10.52±0.07^{n}	11.34±0.07^{w}	78.13±0.01^{b}	2.86±0.01^{x}
Kompolti	10.16±0.01^{o}	13.50±0.01^{r}	76.34±0.01^{g}	3.00±0.01^{w}
Lovrin110	12.76±0.01^{b}	15.71±0.01^{i}	71.53±0.01^{u}	3.60±0.01^{n}
Marina	10.65±0.07^{m}	13.60±0.07^{q}	75.75±0.07^{h}	3.05±0.02^{u}
Monoica	11.38±0.01^{i}	16.78±0.01^{d}	71.84±0.01^{r}	3.85±0.01^{k}
Novosadska	12.46±0.01^{d}	15.90±0.01^{g}	71.64±0.01st	3.80±0.01^{m}
Novosadska+	11.66±0.01^{h}	15.30±0.07^{j}	73.04±0.07^{n}	3.59±0.01^{n}
Santhica 23	10.59±0.11^{mn}	11.23±0.02^{x}	78.18±0.09^{a}	3.54±0.01^{p}
Secuieni jubileu	11.30±0.04^{i}	17.08±0.01^{b}	71.62±0.04^{tu}	4.16±0.04^{f}
Silesia	11.30±0.01^{i}	16.67±0.01^{e}	72.03±0.01^{q}	4.13±0.01^{g}
Simba	11.33±0.01^{i}	14.14±0.01^{n}	74.53±0.01^{l}	3.33±0.01^{r}
Tiborszalassi	10.63±0.05^{mn}	13.91±0.05^{p}	75.46±0.01^{i}	3.41±0.01^{q}
Tisza	11.32±0.03^{i}	15.71±0.03^{hi}	72.97±0.01^{n}	3.97±0.01^{h}
Wojko	12.63±0.02^{c}	14.62±0.01^{k}	72.75±0.02^{o}	4.71±0.01^{a}
Mean ± SD	11.38±0.78	14.47±1.92	74.15±2.48	3.69±0.52
SEM	0.0586	0.0367	0.0480	0.0085
Soybean	15.75±0.07	26.90±0.01	57.35±0.07	9.72±0.13

Σ SFA: Total Saturated fatty acids; Σ MUFA: Total Monounsaturated fatty acids; Σ PUFA: Total polyunsaturated fatty acids. Means with different superscripts are significantly different at P < 0.05.

Palmitoleic (C16:1), γ-linolenic (C18:3 n-6) and eicosadienoic acid (C20:2 n-6), all of them corresponding to the group of unsaturated FA, were all present in hempseeds but not found in soybean. Interestingly, soybean ω6/ω3 ratio was 9.72 which is more than twice higher than the average of all hempseeds.

3.4.3 Amino acid profile

The amino acid profile of hempseeds is reported in Table 5 and Table 6, expressed as total protein basis (g/100 g protein) or whole seed basis (g/100 g seeds) respectively.

Some differences in all the amino acids were found between hempseed varieties ($P<0.05$). The essential amino acids identified in hempseeds were: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine and tryptophan. Among these AAs, hempseed varieties were characterized by high content of leucine that ranged from 6.79 to 7.97 mg/100g protein. However, phenylalanine was not detected in some hempseed varieties and its content ranged between 0.01 to 4.26 mg/100g protein. The non-essential amino acids found were: alanine, arginine, asparagine, cysteine, glutamic acid, glycine, proline, serine and tyrosine. Among these AAs, hempseed varieties were characterized by high content of arginine and glutamine that ranged from 12.66 to 17.50 and 13.04 to 15.49 mg/100g protein, respectively. The content of the sulphur containing amino acids, cystine and methionine ranged from 1.25 to 1.89 and 0.90 to 2.27 mg/100g protein, respectively. From all of the hempseed varieties, Fedora 17 and Tisza showed the highest contents in all amino acids.

Table 5. Amino acid composition (g/100g of protein) of 29 hempseed varieties

Variety	Ala	Arg	Asp	Cys	Glu	Gly	His*	Ile*	Leu*	Lys*	Met*	Phe*	Pro	Ser	Thr*	Trp*	Tyr	Val*
Antal	3.92^{a-d}	15.79^{fg}	9.58^{a-g}	1.58^{d-j}	13.91^{c-f}	3.77^{a-h}	3.41^{e-g}	4.10^{i-k}	7.62^{c-g}	3.75^{b-d}	1.21^{h-m}	3.48^{d}	6.53^{d-f}	4.49^{f-j}	3.12^{c-e}	0.91^{c-f}	2.64^{ij}	4.46^{c-g}
Bacalmas	3.52^{hi}	14.69^{kl}	9.40^{d-j}	1.60^{c-i}	14.21^{b-d}	3.84^{a-f}	3.25^{g-j}	3.91^{kl}	7.10^{i-k}	3.34^{g-j}	1.76^{bc}	0.03^{h}	6.36^{e-h}	4.34^{h-j}	2.93^{e-h}	0.84^{e-g}	2.45^{jk}	4.27^{g-k}
Carmagnola	3.78^{c-g}	15.28^{hi}	9.03^{ml}	1.39^{j-m}	13.08^{hi}	3.62^{g-j}	3.46^{d-g}	4.23^{e-j}	7.50^{e-h}	3.58^{c-g}	1.05^{mn}	2.46^{g}	6.65^{cd}	4.43^{h-j}	2.98^{d-h}	0.86^{c-g}	2.82^{f-i}	4.25^{g-k}
Chameleon	3.61^{f-i}	14.43^{lm}	9.64^{a-e}	1.71^{a-e}	14.23^{b-d}	3.67^{c-j}	3.08^{jk}	4.15^{g-k}	7.15^{i-k}	3.34^{g-j}	1.89^{b}	4.20^{a}	6.64^{c-e}	4.63^{c-h}	2.86^{f-h}	0.87^{c-g}	2.74^{g-j}	4.21^{h-k}
Dioica 88	3.68^{d-i}	16.02^{ef}	9.37^{e-k}	1.61^{c-h}	14.27^{b-d}	3.96^{a}	3.68^{a-d}	4.57^{bc}	7.88^{bc}	3.76^{b-d}	1.65^{cd}	0.01^{h}	6.48^{d-f}	4.72^{b-g}	3.16^{b-e}	0.86^{d-g}	3.26^{a-c}	4.38^{d-j}
Epsilon 88	3.72^{c-g}	12.66^{p}	9.76^{ab}	1.84^{ab}	13.49^{e-i}	3.73^{c-i}	3.36^{f-i}	4.40^{b-g}	7.85^{bc}	3.44^{f-j}	0.90^{n}	0.00^{h}	8.76^{a}	4.43^{h-j}	2.85^{gh}	1.14^{ab}	3.06^{c-f}	4.60^{b-d}
Fedora 17	3.87^{a-e}	17.56^{a}	9.74^{a-c}	1.35^{k-m}	14.47^{bc}	3.82^{a-g}	3.85^{ab}	4.65^{ab}	7.84^{bc}	3.89^{ab}	1.66^{cd}	0.02^{h}	6.88^{bc}	4.88^{a-d}	3.37^{ab}	0.81^{e-g}	3.01^{c-g}	4.73^{ab}
Felina 32	3.58^{g-i}	15.39^{hi}	9.15^{i-m}	1.27^{m}	13.78^{d-g}	3.54^{ij}	3.38^{f-h}	4.14^{g-k}	7.39^{f-i}	3.53^{c-h}	1.03^{mn}	0.03^{h}	5.73^{m-o}	4.30^{ij}	3.06^{c-h}	0.84^{e-g}	2.47^{jk}	4.39^{d-j}
Ferimon FR 8194	3.51^{hi}	16.41^{d}	9.26^{h-l}	1.47^{f-l}	14.24^{b-d}	3.67^{c-j}	3.76^{a-c}	4.57^{bc}	7.67^{b-f}	3.52^{d-i}	2.27^{a}	3.12^{f}	5.39^{pq}	4.61^{d-h}	3.14^{b-e}	0.83^{e-g}	3.42^{ab}	4.42^{c-i}
Fibrol	3.79^{c-g}	17.50^{a}	9.82^{a}	1.55^{e-k}	14.84^{ab}	3.87^{a-d}	3.87^{a}	4.50^{b-e}	7.66^{b-f}	3.96^{ab}	1.42^{e-h}	0.00^{h}	6.45^{d-g}	5.18^{a}	3.41^{a}	0.83^{e-g}	3.18^{b-d}	4.36^{e-k}
Futura 75	3.87^{a-e}	15.53^{gh}	9.21^{i-l}	1.40^{i-m}	13.33^{f-i}	3.65^{e-j}	3.66^{a-d}	4.47^{b-e}	7.61^{c-g}	3.59^{c-f}	1.30^{f-l}	3.06^{f}	6.26^{f-i}	4.56^{e-i}	3.09^{c-g}	0.84^{e-g}	3.21^{b-d}	4.58^{b-e}
Helena	3.59^{g-i}	14.15^{mn}	9.34^{e-l}	1.65^{b-g}	13.72^{d-i}	3.68^{c-j}	3.07^{jk}	3.72^{l}	6.98^{j-l}	3.74^{b-d}	1.35^{f-k}	4.26^{a}	6.57^{de}	4.43^{g-j}	2.94^{e-h}	1.00^{b-d}	2.49^{jk}	3.96^{l}
KC Dora	3.51^{hi}	16.36^{d}	9.23^{i-l}	1.64^{b-g}	14.22^{b-d}	3.87^{a-e}	3.63^{b-e}	4.25^{e-j}	7.21^{h-j}	3.78^{bc}	1.23^{h-m}	3.66^{c}	5.64^{m-p}	4.47^{f-j}	3.07^{c-h}	0.83^{e-g}	2.48^{jk}	4.14^{kl}
KC Virtus	3.46^{i}	15.18^{ij}	9.24^{i-l}	1.89^{a}	13.87^{c-f}	3.68^{c-j}	3.14^{i-k}	4.13^{h-k}	7.58^{c-g}	3.48^{e-j}	1.58^{c-e}	0.00^{h}	5.66^{m-p}	4.23^{j}	2.84^{h}	0.87^{c-g}	3.25^{a-d}	4.19^{i-l}
KC Zuzana	4.04^{ab}	15.76^{fg}	9.23^{i-l}	1.42^{h-m}	13.67^{d-i}	3.74^{b-i}	3.47^{d-g}	4.35^{c-i}	7.97^{b}	3.71^{b-e}	1.14^{k-m}	0.00^{h}	5.82^{l-n}	4.62^{d-h}	3.22^{a-c}	0.79^{fg}	3.16^{b-e}	4.61^{b-d}
Kina	3.94^{a-c}	16.48^{cd}	9.56^{a-h}	1.35^{k-m}	14.52^{bc}	3.89^{a-c}	3.82^{ab}	4.53^{b-d}	7.57^{c-g}	4.11^{a}	1.19^{j-m}	0.03^{h}	7.14^{b}	4.72^{b-g}	3.15^{b-e}	0.85^{d-g}	2.80^{f-i}	4.31^{f-k}
Kompolti	3.80^{b-g}	16.74^{bc}	9.26^{g-l}	1.25^{m}	13.72^{d-i}	3.82^{a-g}	3.85^{ab}	4.56^{b-d}	7.51^{d-h}	3.90^{ab}	1.28^{g-l}	0.00^{h}	5.11^{q}	4.88^{a-d}	3.12^{c-e}	0.82^{e-g}	2.79^{f-i}	4.25^{g-k}
Lovrin110	3.70^{c-i}	15.80^{fg}	9.31^{f-l}	1.63^{c-g}	13.76^{d-h}	3.69^{c-j}	3.45^{d-g}	4.15^{g-k}	7.31^{g-i}	3.74^{b-d}	1.20^{i-m}	0.00^{h}	5.46^{op}	4.41^{h-j}	2.98^{d-h}	0.81^{fg}	2.47^{jk}	4.27^{g-k}
Marina	3.56^{g-i}	14.08^{n}	9.31^{f-l}	1.79^{a-c}	13.96^{c-f}	3.66^{d-j}	3.00^{k}	3.68^{l}	6.86^{kl}	3.24^{j}	1.11^{l-n}	3.89^{b}	5.91^{j-m}	4.43^{h-j}	2.85^{gh}	1.18^{a}	2.48^{jk}	3.98^{l}
Monoica	3.84^{b-f}	14.74^{k}	9.82^{a}	1.55^{e-k}	14.28^{b-d}	3.87^{a-e}	3.42^{e-g}	4.18^{f-j}	7.87^{bc}	3.32^{h-j}	1.16^{k-m}	4.18^{a}	6.73^{cd}	4.81^{b-e}	2.98^{d-h}	1.23^{a}	3.04^{c-f}	4.46^{c-g}
Novosadska	3.63^{e-i}	13.59^{o}	9.06^{k-m}	1.78^{a-d}	13.04^{i}	3.59^{h-j}	3.03^{jk}	4.07^{jk}	6.79^{l}	3.42^{f-j}	1.49^{d-f}	3.31^{e}	6.04^{i-l}	4.25^{j}	2.86^{f-h}	0.97^{c-e}	2.26^{k}	4.18^{j-l}
Novosadska+	3.77^{c-g}	14.89^{jh}	9.14^{j-m}	1.67^{b-f}	13.30^{f-i}	3.64^{f-j}	3.36^{f-i}	4.33^{c-j}	7.53^{d-g}	3.58^{c-g}	1.51^{d-f}	0.00^{h}	6.17^{g-j}	4.37^{h-j}	3.02^{c-h}	0.90^{c-f}	3.14^{b-e}	4.51^{b-f}
Santhica 23	3.48^{hi}	14.96^{jk}	9.15^{i-m}	1.51^{e-k}	13.16^{g-i}	3.48^{j}	3.18^{h-k}	4.14^{h-k}	7.61^{c-g}	3.42^{f-j}	1.16^{k-m}	0.00^{h}	6.28^{f-i}	4.35^{h-j}	2.84^{h}	0.82^{e-g}	3.06^{c-f}	4.42^{c-h}
Secuieni jubileu	3.85^{b-f}	16.30^{de}	9.60^{a-f}	1.35^{k-m}	14.11^{c-e}	3.80^{a-h}	3.59^{c-f}	4.38^{c-h}	7.74^{b-e}	3.61^{c-f}	1.40^{e-j}	2.98^{f}	6.49^{d-f}	4.61^{d-h}	3.09^{c-f}	0.84^{e-g}	2.96^{d-h}	4.46^{c-g}
Silesia	3.48^{hi}	14.15^{mn}	9.42^{d-j}	1.65^{b-g}	13.77^{d-g}	3.85^{a-f}	3.26^{g-j}	4.17^{f-j}	7.15^{i-k}	3.28^{ij}	1.14^{k-m}	0.05^{h}	5.56^{n-p}	4.40^{h-j}	2.83^{h}	1.02^{bc}	2.23^{k}	4.29^{f-k}

continue

Simba	3.79^{b-g}	17.04^{b}	9.43^{c-j}	1.28^{lm}	15.49^{a}	3.85^{a-f}	3.78^{a-c}	4.49^{b-e}	7.62^{c-g}	3.73^{b-e}	1.41^{e-i}	3.09^{f}	6.11^{h-k}	4.75^{b-f}	3.10^{c-f}	0.89^{c-f}	2.87^{e-i}	4.45^{c-g}
Tiborszalassi	3.78^{c-g}	16.78^{bc}	9.46^{b-i}	1.28^{lm}	13.93^{c-f}	3.95^{ab}	3.74^{a-c}	4.43^{b-f}	7.82^{c-d}	4.12^{a}	1.47^{d-g}	0.00^{h}	5.84^{k-n}	4.94^{ab}	3.20^{a-d}	0.72^{g}	2.69^{h-j}	4.62^{bc}
Tisza	3.72^{c-h}	15.81^{fg}	8.89^{m}	1.45^{g-l}	13.29^{f-i}	3.64^{f-j}	3.51^{d-f}	4.30^{d-j}	7.37^{f-i}	3.60^{c-f}	2.14^{a}	2.97^{f}	6.17^{g-j}	4.49^{f-j}	2.96^{d-h}	0.77^{fg}	3.06^{c-f}	4.31^{f-k}
Wojko	4.11^{a}	16.86^{b}	9.71^{a-d}	1.40^{i-m}	14.21^{b-d}	3.78^{a-h}	3.51^{d-f}	4.87^{a}	8.34^{a}	3.37^{f-j}	1.50^{d-f}	0.01^{h}	6.91^{bc}	4.93^{a-c}	3.22^{a-c}	0.82^{e-g}	3.50^{a}	4.87^{a}
Mean ± SD	3.72±0.19	15.55±1.19	9.38±0.27	1.53±0.19	13.93±0.59	3.74±0.14	3.47±0.28	4.29±0.28	7.52±0.36	3.62±0.25	1.40±0.33	1.55±1.76	6.37±0.69	4.57±0.25	3.04±0.18	0.90±0.13	2.86±0.36	4.38±0.22
SEM	0.12	0.15	0.15	0.10	0.34	0.11	0.11	0.13	0.15	0.12	0.11	0.08	0.14	0.14	0.12	0.08	0.14	0.11
Soybean	3.71	10.98	8.95	0.74	12.43	3.44	3.52	4.22	8.49	5.67	1.37	4.72	8.70	4.30	3.42	1.13	3.46	5.13

*Essential amino acids. Ala: alanine; Arg: arginine; Asp: asparagine; Cys: cysteine; Glu: glutamine; Gly: glycine; His: histidine; Ile: isoleucine; Leu: leucine; Lys: lysine; Met: methionine; Phe: phenylalanine; Pro: proline; Ser: serine; Thr: threonine; Trp: tryptophan; Tyr: tyrosine; Val: valine. Means with different superscripts are significantly different at $P < 0.05$.

Table 6. Amino acid composition (g/100g of seed) of 29 hempseed varieties

Variety	Ala	Asp	Arg	Cys	Glu	Gly	His*	Ile*	Leu*	Lys*	Met*	Phe*	Pro	Ser	Thr*	Trp*	Tyr	Val*
Antal	0.84^{f-i}	2.05^{mn}	3.38^{m}	0.34^{d-h}	2.98^{ml}	0.81^{ij}	0.73^{k-n}	0.88^{k-m}	1.63^{jk}	0.80^{h-k}	0.26^{hi}	0.75^{e-g}	1.40^{hi}	0.96^{i-k}	0.67^{i-l}	0.20^{d-g}	0.56^{m-o}	0.95^{i-l}
Bacalmas	0.83^{g-j}	2.22^{g-i}	3.46^{k}	0.38^{a-e}	3.35^{d-h}	0.91^{c-g}	0.77^{i-k}	0.92^{i-l}	1.67^{h-j}	0.79^{i-l}	0.41^{bc}	0.01^{j}	1.50^{g}	1.02^{g-i}	0.69^{f-j}	0.20^{d-g}	0.58^{l-n}	1.01^{f-i}
Carmagnola	0.94^{a-c}	2.25^{e-h}	3.81^{hi}	0.35^{c-g}	3.27^{f-j}	0.90^{c-g}	0.86^{d-g}	1.06^{c-e}	1.87^{b-d}	0.89^{b-e}	0.26^{hi}	0.61^{i}	1.66^{b}	1.11^{ef}	0.74^{b-g}	0.21	0.70^{e-h}	1.06^{d-f}
Chameleon	0.86^{d-h}	2.31^{b-e}	3.45^{kl}	0.41^{a}	3.40^{c-f}	0.88^{d-g}	0.74^{j-m}	0.99^{e-h}	1.71^{g-i}	0.80^{h-l}	0.45^{b}	1.00^{a}	1.59^{c-e}	1.11^{de}	0.68^{g-k}	0.21^{c-e}	0.65^{g-j}	1.01^{f-i}
Dioica 88	0.86^{e-h}	2.20^{h-k}	3.76^{i}	0.38^{a-d}	3.35^{d-h}	0.93^{a-d}	0.86^{d-g}	1.07^{b-d}	1.85^{b-e}	0.88^{d-f}	0.39^{cd}	0.00^{j}	1.52^{fg}	1.11^{ef}	0.74^{b-g}	0.20^{d-g}	0.76^{b-e}	1.03^{e-g}
Epsilon 88	0.78^{jk}	2.03^{mn}	2.64^{q}	0.38^{a-d}	2.81^{n}	0.78^{j}	0.70^{l-o}	0.92^{j-l}	1.63^{jk}	0.72^{m-o}	0.19^{j}	0.00^{j}	1.82^{a}	0.92^{k}	0.59^{m}	0.24^{a-c}	0.64^{i-l}	0.96^{i-k}
Fedora 17	0.94^{a-c}	2.37^{ab}	4.27^{a}	0.33^{e-h}	3.52^{a-c}	0.93^{a-d}	0.94^{a-c}	1.13^{ab}	1.91^{ab}	0.95^{ab}	0.40^{bc}	0.00^{j}	1.67^{b}	1.19^{a-c}	0.82^{a}	0.20^{e-g}	0.73^{d-f}	1.15^{ab}
Felina 32	0.84^{f-i}	2.14^{j-l}	3.60^{j}	0.30^{h}	3.22^{h-j}	0.83^{h-j}	0.79^{h-j}	0.97^{g-j}	1.73^{gh}	0.83^{f-i}	0.24^{ij}	0.01^{j}	1.34^{i-k}	1.01^{h-j}	0.72^{e-i}	0.21^{d-f}	0.58^{k-n}	1.02^{e-h}
Ferimon FR 8194	0.87^{d-h}	2.30^{b-e}	4.07^{c}	0.37^{a-f}	3.53^{a-c}	0.91^{c-f}	0.93^{a-c}	1.13^{ab}	1.90^{bc}	0.87^{ef}	0.56^{a}	0.77^{de}	1.34^{i-l}	1.14^{b-e}	0.78^{a-d}	0.21^{d-f}	0.85^{a}	1.10^{b-d}
Fibrol	0.90^{b-f}	2.33^{a-d}	4.15^{b}	0.37^{a-f}	3.52^{a-c}	0.92^{b-e}	0.92^{a-d}	1.07^{cd}	1.82^{d-f}	0.94^{a-c}	0.34^{d-f}	0.00^{j}	1.53^{e-g}	1.23^{a}	0.81^{a}	0.20^{e-g}	0.75^{c-e}	1.03^{e-g}
Futura 75	0.96^{ab}	2.28^{c-g}	3.84^{gh}	0.35^{b-g}	3.30^{e-i}	0.90^{c-g}	0.91^{b-d}	1.11^{a-c}	1.88^{b-d}	0.89^{c-e}	0.32^{e-g}	0.76^{ef}	1.55^{e-g}	1.13^{c-e}	0.76^{a-e}	0.21^{d-f}	0.79^{a-d}	1.13^{a-c}
Helena	0.83^{g-j}	2.17^{i-k}	3.28^{n}	0.38^{a-d}	3.18^{i-k}	0.85^{g-i}	0.71^{k-n}	0.86^{lm}	1.62^{jk}	0.87^{e-g}	0.31^{e-g}	0.99^{a}	1.52^{e-g}	1.03^{g-i}	0.68^{h-k}	0.23^{a-d}	0.58^{l-n}	0.92^{j-l}
KC Dora	0.81^{h-k}	2.14^{j-l}	3.79^{hi}	0.38^{a-d}	3.30^{e-i}	0.90^{c-g}	0.84^{f-h}	0.98^{f-i}	1.67^{h-j}	0.88^{ef}	0.28^{f-i}	0.85^{b}	1.31^{j-m}	1.03^{f-h}	0.71^{e-i}	0.20^{e-g}	0.57^{l-n}	0.96^{i-k}
KC Virtus	0.75^{k}	2.00^{mn}	3.30^{n}	0.41^{a}	3.01^{lm}	0.80^{j}	0.68^{m-o}	0.90^{k-m}	1.65^{i-k}	0.75^{k-m}	0.34^{de}	0.00^{j}	1.23^{n}	0.92^{k}	0.62^{lm}	0.19^{e-g}	0.71^{e-h}	0.91^{k-m}
KC Zuzana	0.88^{d-g}	2.00^{n}	3.42^{k-m}	0.31^{gh}	2.97^{l-n}	0.81^{ij}	0.75^{i-l}	0.94^{h-k}	1.73^{gh}	0.81^{h-k}	0.25^{i}	0.00^{j}	1.26^{mn}	1.00^{h-j}	0.70^{f-j}	0.17^{g}	0.68^{f-i}	1.00^{g-i}
Kina	0.92^{b-e}	2.23^{f-i}	3.85^{gh}	0.32^{gh}	3.39^{c-g}	0.91^{c-f}	0.89^{b-f}	1.06^{cd}	1.77^{fg}	0.96^{a}	0.28^{g-i}	0.01^{j}	1.67^{b}	1.10^{ef}	0.74^{c-h}	0.20^{d-g}	0.66^{g-j}	1.01^{f-i}
Kompolti	0.96^{ab}	2.34^{a-c}	4.24^{a}	0.32^{gh}	3.47^{a-d}	0.97^{ab}	0.97^{a}	1.15^{a}	1.90^{bc}	0.99^{a}	0.32^{e-g}	0.00^{j}	1.29^{k-n}	1.24^{a}	0.79^{a-c}	0.21^{d-f}	0.71^{e-h}	1.08^{c-e}

continue

Lovrin110	0.92^{b-d}	2.32^{a-e}	3.94^{ef}	0.41^{a}	3.44^{b-e}	0.92^{b-e}	0.86^{d-g}	1.04^{d-g}	1.82^{d-f}	0.93^{a-d}	0.30^{e-h}	0.00^{j}	1.36^{h-j}	1.10^{ef}	0.74^{b-f}	0.21^{d-f}	0.62^{j-m}	1.07^{de}
Marina	0.76^{k}	2.00^{n}	3.03^{p}	0.38^{a-d}	3.00^{lm}	0.79^{j}	0.64^{o}	0.79^{n}	1.48^{l}	0.70^{no}	0.24^{ij}	0.84^{bc}	1.27^{l-n}	0.95^{jk}	0.61^{m}	0.26^{a}	0.53^{no}	0.85^{m}
Monoica	0.77^{jk}	1.98^{n}	2.97^{p}	0.31^{gh}	2.88^{mn}	0.78^{j}	0.69^{m-o}	0.84^{mn}	1.59^{k}	0.67^{o}	0.23^{ij}	0.85^{b}	1.36^{h-j}	0.97^{h-k}	0.60^{m}	0.25^{ab}	0.61^{j-m}	0.90^{lm}
Novosadska	0.81^{h-k}	2.01^{mn}	3.02^{p}	0.40^{ab}	2.90^{mn}	0.80^{j}	0.67^{no}	0.91^{j-m}	1.51^{l}	0.76^{j-m}	0.33^{e-g}	0.74^{f-h}	1.34^{i-k}	0.94^{jk}	0.63^{k-m}	0.22^{b-e}	0.51^{o}	0.93^{j-l}
Novosadska+	0.86^{e-h}	2.08^{lm}	3.38^{lm}	0.38^{a-e}	3.02^{k-m}	0.83^{h-j}	0.76^{i-k}	0.98^{f-i}	1.71^{g-i}	0.81^{g-j}	0.34^{de}	0.00^{j}	1.40^{hi}	0.99^{h-k}	0.69^{g-k}	0.21^{d-f}	0.71^{e-g}	1.03^{e-g}
Santhica 23	0.90^{b-e}	2.38^{a}	3.89^{fg}	0.39^{ab}	3.42^{b-f}	0.90^{c-g}	0.83^{gh}	1.07^{b-d}	1.98^{a}	0.89^{c-e}	0.30^{e-h}	0.00^{j}	1.63^{b-d}	1.13^{c-e}	0.74^{c-h}	0.22^{b-e}	0.80^{a-d}	1.15^{ab}
Secuieni jubileu	0.91^{b-e}	2.26^{d-h}	3.84^{gh}	0.32^{f-h}	3.33^{d-i}	0.90^{c-g}	0.85^{e-h}	1.03^{d-g}	1.83^{c-f}	0.85^{e-h}	0.33^{e-g}	0.70^{h}	1.53^{e-g}	1.09^{e-g}	0.73^{d-h}	0.20^{e-g}	0.70^{e-i}	1.05^{d-g}
Silesia	0.79^{i-k}	2.13^{kl}	3.21^{o}	0.37^{a-e}	3.12^{j-l}	0.87^{e-h}	0.74^{j-m}	0.95^{h-k}	1.62^{jk}	0.74^{l-n}	0.26^{hi}	0.01^{j}	1.26^{mn}	1.00^{h-j}	0.64^{j-m}	0.23^{a-d}	0.51^{o}	0.97^{h-j}
Simba	0.88^{c-g}	2.20^{h-k}	3.97^{de}	0.30^{h}	3.61^{a}	0.90^{c-g}	0.88^{c-f}	1.05^{c-f}	1.77^{e-g}	0.87^{e-g}	0.33^{e-g}	0.72^{gh}	1.42^{h}	1.11^{de}	0.72^{e-i}	0.21^{c-e}	0.67^{f-j}	1.04^{e-g}
Tiborszalassi	0.90^{b-e}	2.26^{d-h}	4.02^{cd}	0.31^{gh}	3.33^{d-i}	0.95^{a-c}	0.90^{b-e}	1.06^{cd}	1.87^{b-d}	0.99^{a}	0.35^{de}	0.00^{j}	1.40^{hi}	1.18^{a-d}	0.76^{a-e}	0.18^{fg}	0.64^{h-k}	1.11^{a-d}
Tisza	1.00^{a}	2.39^{a}	4.25^{a}	0.39^{a-c}	3.57^{ab}	0.98^{a}	0.94^{ab}	1.16^{a}	1.98^{a}	0.97^{a}	0.57^{a}	0.80^{cd}	1.66^{bc}	1.21^{ab}	0.80^{ab}	0.21^{d-f}	0.82^{ab}	1.16^{a}
Wojko	0.94^{a-c}	2.21^{g-j}	3.84^{gh}	0.32^{f-h}	3.24^{g-j}	0.86^{f-i}	0.80^{hi}	1.11^{a-c}	1.90^{bc}	0.77^{i-m}	0.34^{de}	0.00^{j}	1.57^{d-f}	1.12^{c-e}	0.73^{c-h}	0.19^{e-g}	0.80^{a-c}	1.11^{a-d}
Mean ± SD	0.87± 0.07	2.19± 0.13	3.64± 0.42	0.36± 0.04	3.26± 0.23	0.88± 0.06	0.81± 0.10	1.00± 0.10	1.76± 0.14	0.85± 0.09	0.33± 0.09	0.36± 0.41	1.46± 0.16	1.07± 0.10	0.71± 0.07	0.21± 0.02	0.67± 0.10	1.02± 0.08
SEM	0.03	0.04	0.04	0.02	0.08	0.02	0.03	0.03	0.04	0.03	0.03	0.02	0.03	0.03	0.03	0.02	0.03	0.03
Soybean	1.39	3.37	4.11	0.28	4.68	1.29	1.33	1.93	3.19	2.13	0.52	1.78	3.27	1.62	1.29	0.43	1.30	1.59

*Essential amino acids. Ala: alanine; Arg: arginine; Asp: asparagine; Cys: cysteine; Glu: glutamine; Gly: glycine; His: histidine; Ile: isoleucine; Leu: leucine; Lys: lysine; Met: methionine; Phe: phenylalanine; Pro: proline; Ser: serine; Thr: threonine; Trp: tryptophan; Tyr: tyrosine; Val: valine. Means with different superscripts are significantly different at $P < 0.05$.

3.4.4 Mineral composition

Contents of some minerals known to be essential in human and animal nutrition are shown in Table 7. Some differences in all the mineral contents were found between hempseed varieties ($P<0.05$). Sodium was analyzed but was found not detectable (<0.5 mg/100g sample) in all the samples, so it is not presented in the results. Helena was the most outstanding variety in terms of mineral content. Compared to soybean, hempseed varieties showed similar or greater amounts in most of the minerals evaluated except of calcium. Content of calcium was much higher in soybean (294.17) than in hempseeds (130.57). Manganese and sodium were the two minerals found no detectable in soybean.

3.4.5 Cannabinoid content

The cannabinoid content (µg/g) of 29 varieties of hempseeds is shown in Table 8. Some differences in the three types of cannabinoids were found between hempseed varieties ($P < 0.05$). They showed average CBD, THC and CBN contents (µg/g) of 39.54, 7.83 and 18.31 respectively. CBD was present in all hempseed varieties, while CBN and THC was found not detectable in some of them. From the 29 varieties analyzed, all of them were under the 0.02% limit value of THC [5]. In fact, only 5 varieties contained THC ranging from 15.21 - 163.33 µg/g equals to 0.0015 - 0.0163%.

Table 7. Mineral composition (mg/100g) of 29 hempseed varieties

	Ca	Mg	K	Fe	Mn	Cu	Zn
Antal	153.76±8.01^{a-c}	376.19±6.29^{d-h}	976.28±29.61^{a-e}	32.37±7.21^{a}	10.51±0.23^{bc}	1.13±0.01^{c-f}	4.56±0.01^{f-j}
Bacalmas	149.72±1.68^{b-e}	347.20±7.87^{f-j}	1084.62±36.27^{ab}	20.49±1.55^{bc}	8.85±0.24^{c-g}	0.92±0.01^{fg}	3.09±0.28^{l-n}
Carmagnola	113.07±1.90^{i-l}	321.14±9.43^{h-k}	1041.25±13.69^{a-d}	16.58±0.05^{b-d}	8.01±0.24^{d-h}	1.16±0.01^{c-e}	3.05±0.31^{l-n}
Chameleon	137.15±13.32^{c-i}	288.98±19.32^{jk}	917.01±37.36^{a-f}	14.76±1.11^{c-f}	9.56±0.01^{b-f}	1.02±0.05^{e-g}	4.11±1.18^{g-l}
Dioica 88	157.87±17.95^{a-c}	421.53±16.35^{b-e}	590.25±31.76^{g-j}	8.11±1.01^{d-g}	9.78±0.06^{b-d}	1.27±0.16^{a-d}	7.02±1.21^{ab}
Epsilon 88	171.04±3.26^{ab}	413.43±6.07^{b-f}	538.96±34.95^{j}	9.37±0.33^{d-g}	8.63±0.05^{d-h}	0.90±0.04^{g}	4.90±0.06^{e-h}
Fedora 17	125.47±3.07^{e-k}	298.57±41.80^{i-k}	854.86±21.53^{b-i}	6.98±0.88^{e-g}	8.02±0.16^{d-h}	1.21±0.06^{b-e}	4.47±0.02^{f-k}
Felina 32	133.77±8.06^{c-j}	297.43±13.26^{i-k}	934.01±51.52^{a-f}	14.31±2.96^{c-g}	6.12±0.54^{i-k}	1.00±0.03^{e-g}	3.08±0.24^{l-n}
Ferimon FR8194	111.53±2.42^{j-l}	360.03±4.18^{e-i}	872.52±105.82^{b-h}	16.09±8.65^{c-e}	4.79±0.08^{k}	1.01±0.18^{e-g}	3.10±0.27^{l-n}
Fibrol	113.56±16.68^{i-l}	375.36±4.26^{d-h}	1182.65±557.73^{a}	25.68±6.50^{ab}	10.66±0.76^{a-c}	1.05±0.07^{d-g}	4.70±1.36^{f-i}
Futura 75	99.61±14.03^{l}	305.79±68.42^{i-k}	780.63±250.73^{c-j}	14.61±3.37^{c-f}	2.84±0.02^{l}	0.99±0.24^{e-g}	3.06±0.0.30^{l-n}
Helena	177.21±7.55^{a}	426.46±2.84^{b-e}	740.08±38.75^{e-j}	8.46±2.68^{d-g}	9.55±0.31^{b-g}	1.47±0.06^{a}	6.33±0.89^{b-d}
KC Dora	119.01±9.51^{g-l}	499.90±5.72^{a}	744.45±23.62^{d-j}	5.85±0.02^{fg}	9.41±0.40^{c-g}	1.10±0.40^{c-g}	5.58±0.34^{c-f}
KC Virtus	153.04±15.05^{a-d}	463.03±54.20^{ab}	578.33±13.27^{h-j}	6.46±0.42^{fg}	10.54±1.25^{bc}	1.12±0.18^{c-g}	5.93±0.28^{b-e}
KC Zuzana	151.47±0.11^{b-d}	328.92±0.89^{g-k}	1050.04±21.33^{a-c}	14.39±0.02^{c-g}	8.19±0.09^{d-h}	1.30±0.01^{a-c}	3.29±0.01^{k-n}
Kina	70.99±22.02^{m}	301.31±24.04^{i-k}	665.84±232.85^{f-j}	16.27±12.93^{b-e}	5.34±1.22^{jk}	0.57±0.01^{h}	4.96±0.01^{e-g}
Kompolti	128.73±4.71^{d-k}	470.11±11.25^{ab}	652.69±102.91^{f-j}	6.62±1.13^{fg}	9.60±0.19^{b-e}	1.20±0.16^{b-e}	5.10±1.49^{e-g}
Lovrin110	119.23±5.06^{g-l}	446.57±5.04^{a-c}	649.96±31.97^{f-j}	5.06±0.61^{g}	9.62±0.67^{b-e}	1.17±0.10^{b-e}	3.49±0.29^{j-m}
Marina	139.80±22.54^{c-g}	384.06±39.80^{c-h}	602.14±47.21^{g-j}	8.75±0.70^{d-g}	8.83±0.87^{c-g}	1.00±0.18^{e-g}	2.21±0.62^{n}
Monoica	133.60±3.00^{c-j}	362.31±21.99^{e-i}	878.12±17.02^{b-g}	19.29±2.55^{bc}	8.30±0.04^{d-h}	1.18±0.02^{b-e}	4.56±0.03^{f-j}
Novosadska	122.97±24.62^{g-l}	391.63±14.55^{c-g}	593.55±149.77^{g-j}	11.96±5.04^{c-g}	7.75±2.89^{f-i}	1.15±0.16^{c-e}	7.93±0.79^{a}
Novosadska+	138.98±27.86^{c-h}	274.66±84.25^{k}	793.87±105.81^{b-j}	14.31±13.57^{c-g}	5.81±1.96^{jk}	0.91±0.01^{fg}	2.88±0.02^{mn}
Santhica 23	126.18±1.63^{e-k}	329.62±1.61^{g-k}	997.12±16.51^{a-e}	9.66±0.68^{d-g}	7.79±0.04^{e-i}	1.05±0.01^{d-g}	5.15±0.32^{d-g}
Secuieni jubileu	110.12±0.89^{j-l}	441.95±25.79^{a-d}	601.72±13.63^{g-j}	9.34±2.56^{d-g}	11.35±0.29^{ab}	1.12±0.21^{c-g}	3.72±0.03^{h-m}
Silesia	148.25±6.43^{b-f}	328.13±7.41^{g-k}	997.58±28.66^{a-e}	14.26±0.75^{c-g}	7.71±0.45^{g-i}	1.08±0.01^{c-g}	3.53±0.30^{i-m}
Simba	123.47±10.56^{f-l}	347.45±9.44^{f-j}	805.21±329.11^{b-j}	13.21±4.22^{c-g}	6.93±1.41^{h-j}	1.04±0.17^{e-g}	3.09±0.32^{l-n}
Tiborszalassi	107.83±2.37^{kl}	322.80±80.12^{g-k}	993.30±29.36^{a-e}	11.63±0.89^{c-g}	12.48±0.34^{a}	1.08±0.10^{c-g}	5.38±0.03^{d-f}
Tisza	115.00±10.58^{h-l}	436.52±70.13^{a-d}	568.26±47.18^{ij}	7.53±2.32^{d-g}	9.20±1.84^{c-g}	1.15±0.10^{c-e}	5.99±0.26^{b-e}
Wojko	134.06±6.56^{c-j}	376.48±21.44^{d-h}	509.96±2.33^{j}	8.10±0.35^{d-g}	7.78±0.41^{e-i}	1.40±0.11^{ab}	6.70±0.22^{bc}

continue

Mean ± SD	130.57±23.80	370.26±65.39	799.84±216.50	12.78±6.95	8.41±2.14	1.09±0.18	4.52±1.48
SEM	12.12	33.84	145.23	4.62	0.91	0.11	0.58
Soybean	294.17±51.81	347.02±1.32	739.19±266.04	12.15±5.06	n.d	0.67±0.02	2.67±0.32

Ca: calcium; Mg: magnesium; K: potassium, Fe: iron; Mn: manganese; Cu: copper; Zn: zinc. Means with different superscripts are significantly different at $P<0.05$.

Table 8. Cannabinoid content (µg/g) in 29 hempseed varieties

	CBD	THC	CBN
Antal	73.24±5.81^{bc}	15.21±5.46^{b}	16.40±4.56^{bc}
Bacalmas	94.15±7.77^{a}	n.d	11.56±1.08^{cd}
Carmagnola	29.42±3.57$^{j\text{-}m}$	n.d	18.98±10.15^{bc}
Chameleon	32.45±6.41$^{i\text{-}l}$	n.d	15.23±4.99$^{b\text{-}d}$
Dioica 88	76.72±4.10^{b}	n.d	16.23±6.57^{bc}
Epsilon 88	64.66±4.62^{cd}	n.d	15.71±5.54^{bc}
Fedora 17	18.39±3.62^{no}	n.d	n.d
Felina 32	59.58±1.49$^{d\text{-}f}$	16.71±5.52^{b}	12.68±3.54^{cd}
Ferimon FR8194	7.86±0.60^{pq}	n.d	16.12±6.75^{bc}
Fibrol	22.11±4.10^{mn}	n.d	n.d
Futura 75	8.90±0.67^{pq}	n.d	n.d
Helena	50.88±3.18^{fg}	n.d	17.57±4.97^{bc}
KC Dora	9.78±0.26$^{o\text{-}q}$	n.d	n.d
KC Virtus	52.93±7.36^{ef}	n.d	15.86±5.35^{bc}
KC Zuzana	23.51±6.32$^{l\text{-}n}$	n.d	10.63±0.91^{cd}
Kina	41.46±3.98^{h}	163.33±45.60^{a}	221.89±33.00^{a}
Kompolti	23.25±3.55^{mn}	n.d	n.d
Lovrin110	43.19±6.32^{gh}	n.d	17.06±5.31^{bc}
Marina	28.14±3.66$^{k\text{-}m}$	n.d	n.d
Monoica	35.10±6.20$^{h\text{-}k}$	n.d	15.50±5.83^{bc}
Novosadska	91.88±4.60^{a}	n.d	16.52±6.30^{bc}
Novosadska+	37.99±5.33$^{h\text{-}j}$	15.83±5.78^{b}	29.19±3.89^{b}
Santhica 23	5.66±0.90^{q}	n.d	n.d
Secuieni jubileu	61.08±3.45^{de}	n.d	17.71±4.16^{bc}
Silesia	20.50±1.04^{mn}	n.d	n.d
Simba	32.41±3.83$^{j\text{-}l}$	15.97±6.88^{b}	11.64±1.16^{cd}
Tiborszalassi	41.72±3.59^{h}	n.d	17.63±5.99^{bc}
Tisza	43.51±2.24^{gh}	n.d	17.00±6.90^{bc}
Wojko	16.23±4.94^{no}	n.d	n.d
Mean ± SD	39.54±24.33	7.83±30.79	18.31±40.01
SEM	4.41	8.75	7.53

CBD: cannabidiol; THC: tetrahydrocannabidiol; CBN: cannabinol. n.d: not detected, < 5.17 µg/g. Means with different superscripts are significantly different at P<0.05.

3.5 Discussion

3.5.1 Chemical composition

In general, the hempseed chemical composition is consistent with previous studies in which the variety and agronomic conditions affected the composition of hempseeds [19, 20, 21]. The analysis of variance (ANOVA) in this study showed significant differences (P<0.05) in the dry matter, crude protein, ether extract and ash contents among the 29 hempseed varieties analyzed. Results showed that whole hempseeds contain in average 92.83% of dry matter, which is consistent with previous studies that reported values between 91.2 to 96.21% [22,23,24,25,26,27]. This could be due to environmental and storage conditions differences [20]. The soybean used had a lower dry matter content (89.91%) compared to the 29 varieties of hempseed, probably due to the differences in agronomical practices and storage. Protein content

(% of DM) in whole hempseeds was 25.17, this value is high and fits in the wide range of 12.2 to 28 sourced from different studies [19,20,22,23,24,25,26,28,29]. In the case of soybean, that has been always considered as a rich source of protein, the crude protein quantity (41.79%) was notably higher. Fat content (% of DM) was the most abundant fraction in whole hempseeds representing an average of 31.72. This value is high if we consider the wide range of 9.31 to 35.00 found in the literature [27,19,20,22,23,24,25,26,28,30] and also in comparison to the fat content found in the soybean (18.65). Ash content (% of DM) of hempseeds was 5.54 and also seems to be consistent with previous works that reported values between 4.4 to 7.20 [19,23,24,26,29,30] and close to the content found in soybean (5.20).

The chemical composition of some of the hempseed varieties evaluated were found in the literature as whole seeds, this is why the content of crude protein (Figure 2) and fat (Figure 3) could be compared. Crude protein was higher in comparison to all the other authors. Alonso-Esteban et al., 2022 [27] found lower values for CP in all the varieties mostly because a lower nitrogen-protein conversion factor (5.3) was used. Fat content was almost similar to other authors. For Fedora 17 v., a wide variation was found among the literature.

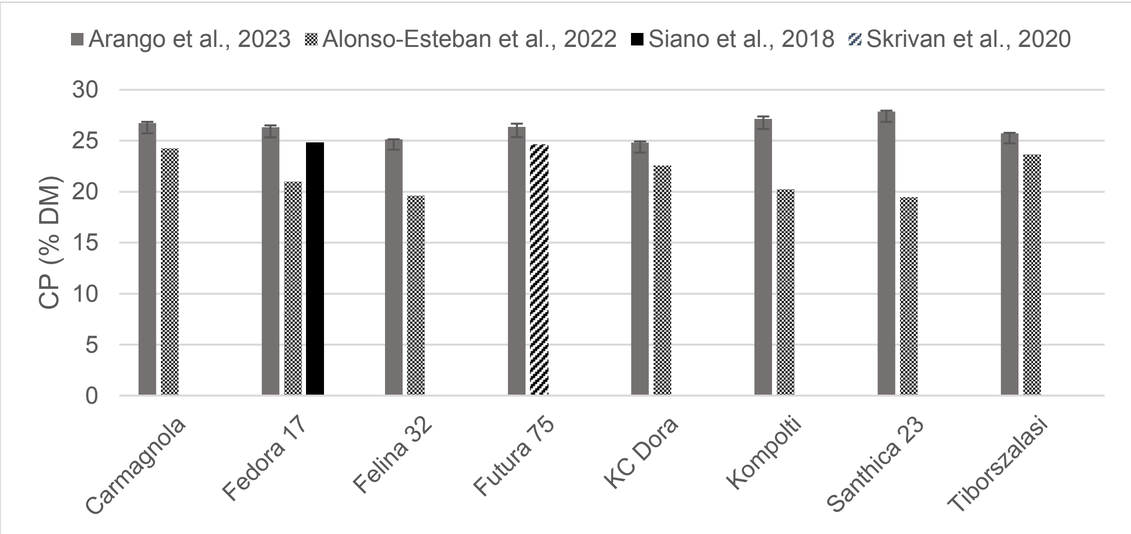

Figure 2. Content of crude protein (% DM) in whole hempseeds of Carmagnola, Fedora 17, Felina 32, Futura 75, KC Dora, Kompolti, Santhica 23 and Tiborszalassi varieties in comparison with the literature [27,34,46].

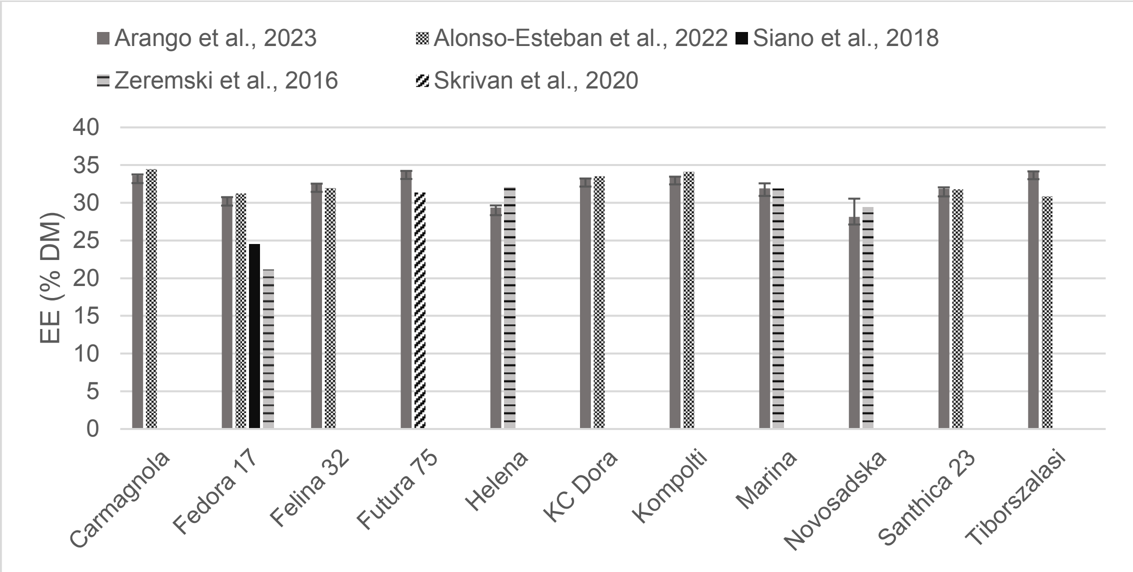

Figure 3. Content of fat (% DM) in whole hempseeds of Carmagnola, Fedora 17, Felina 32, Futura 75, Helena, KC Dora, Kompolti, Marina, Novosadska, Santhica 23 and Tiborszalassi varieties in comparison with the literature [27,31,34,46].

3.5.2 Fatty acid profile

The FA profile of hempseeds reported in this work is consistent with those of other authors. All of them and also this current work reported linoleic acid (C18:2n-6) as the major FA, followed by α-linolenic acid (C18:3n-3) or oleic acid (C18:1) [22,25, 26,28,30,31,32,33,34,35,36,37]. In addition, all the studies concluded that FA composition is highly influenced by the genotype [37,38] which is in agreement with this study where a significant variation in the FA profile between hempseed varieties was found ($P < 0.05$). In overall, FA composition of 29 hempseed varieties revealed a distribution of SFA, MUFA and PUFA of 10.07–12.95, 11.23–17.93 and 70.74–78.17% of FAME respectively. The values of SFA were higher than the values reported in the literature, whereas the MUFA and PUFA were according to previous studies (SFA: 8.24, 8.60, 9.33 and 9.34; MUFA: 9.37, 11.16, 14.56, 16.05 and 18.70; PUFA: 71.98, 72.58, 74.78, 76.4 and 77.7) [25,26,28,32,33]. In comparison to soybean, the FA distribution of hempseeds is higher in PUFA, and therefore lower in SFA and MUFA. This was mostly because soybean lacks of γ-linolenic and C20:2n-6, not to mention the fact that its content of α-linolenic is almost 3 times less than in hempseeds. The ω6/ω3 ratio of all the hempseed varieties were less than the established 3:1 to 5:1 by the European Food and Safety Authority (EFSA) that ensures the maintenance of an optimal state of health in humans. In contrast, this ratio (9.72) was much higher in soybean mostly because its low content (5.35) of C18:3n3.

Individual comparisons of FA profile (% of FAME) could be done for some of the hempseed varieties studied on previous works (Figures 4-6) in which total content of SFA, MUFA and PUFA are according to the values herein reported [19,31,33,34].

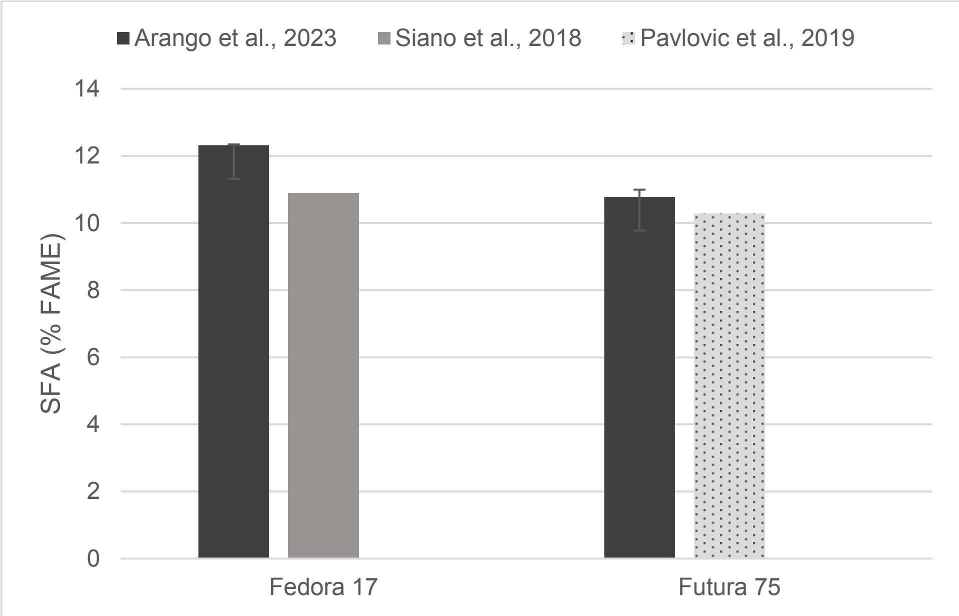

Figure 4. Content of saturated fatty acids (% of FAME) in seeds of Fedora 17 and Futura 75 varieties in comparison with the literature [33, 34].

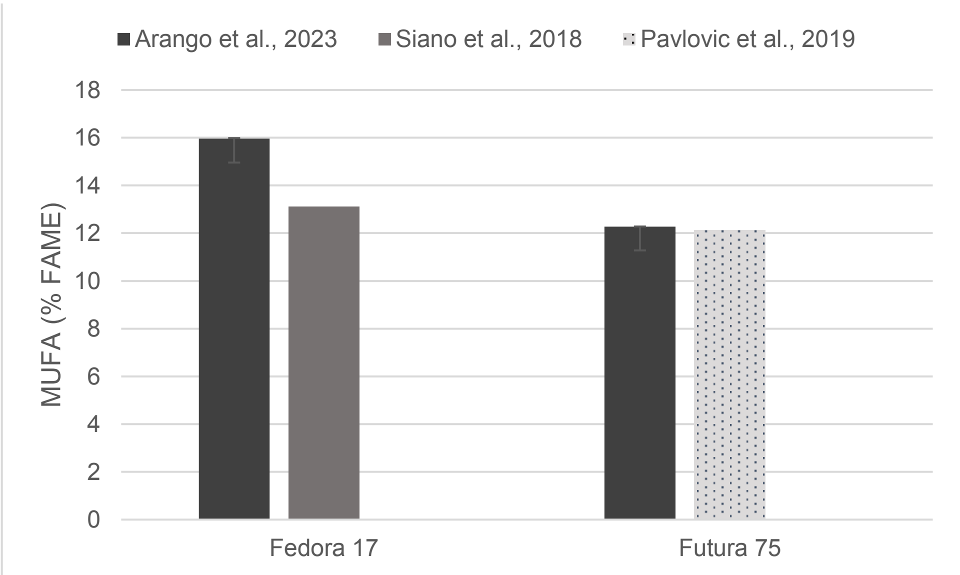

Figure 5. Content of mono unsaturated fatty acids (% of FAME) in seeds of Fedora 17 and Futura 75 varieties in comparison with the literature [33, 34].

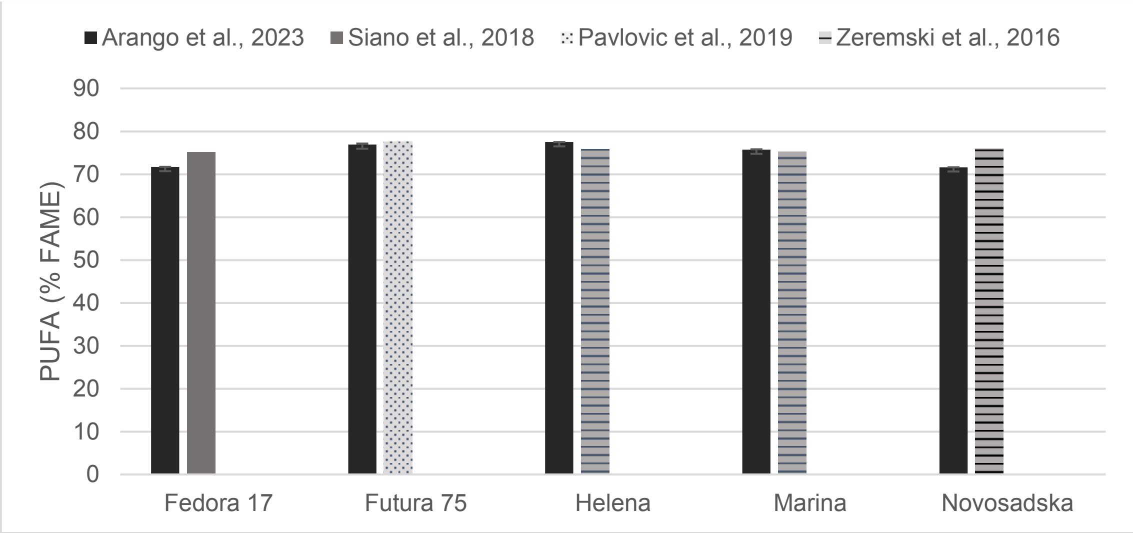

Figure 6. Content of poly unsaturated fatty acids (% of FAME) in seeds of Fedora 17, Futura 75, Helena, Marina and Novosadska varieties in comparison with the literature [31,33, 34].

3.5.3 Amino acid profile

First of all, it is evident that the way of expressing the amino acid content needs to be considered in order to make comparisons or state nutritional requirement fulfillments. The way on how the source of protein is going to be assumed by the consumer needs to be specified. Our results showed that hempseeds and soybean seem to have a similar amino acid profile when they are expressed in terms of total protein (Table 5), but when it comes to report it in whole seed basis (Table 6) soybean clearly has an advantage because of its higher protein content. So, if the purpose is to use protein isolates as food ingredients is accurate to consider the amount of amino acid expressed in protein. On the other hand, hempseeds when used as natural food ingredient in a normal based diet are better to be compared as seeds.

In general, our results are consistent with previous studies in which the variety and agronomic conditions affected the amino acid profile. The amino acid composition of hempseeds showed that they contain all the essential amino acids (EAAs) for human health as many others authors had already reported [4,20,21,23,30,36,37,39,40]. Hempseed presents very high levels of arginine and glutamic acid and a scarce content of lysine [37]. In fact, this study identified arginine (3.64 g/100g seed) as the main amino acid, followed by glutamic acid (3.26 g/100g seed). This represents an interesting fact because arginine has been known for its beneficial properties like ammonia detoxification, fetal growth enhancing, and insulin resistance reduction [37].

In comparison to soybean, all EAAs were higher (Figure 7). In fact, lysine would be the first limiting amino acid of hempseeds (2.13 VS 0.85 g/100g seed) which has been already stated in the literature [20,41,42,43]. Both hempseed and soybean are similarly low in sulfur containing amino acids. Considering the average of the EAAs content of the 29 whole hempseed varieties and a serving size of 30 g, hempseeds fulfilled around 20 percent of a 70 kg adult daily requirement suggested by the FAO/WHO (Figure 8) [44].

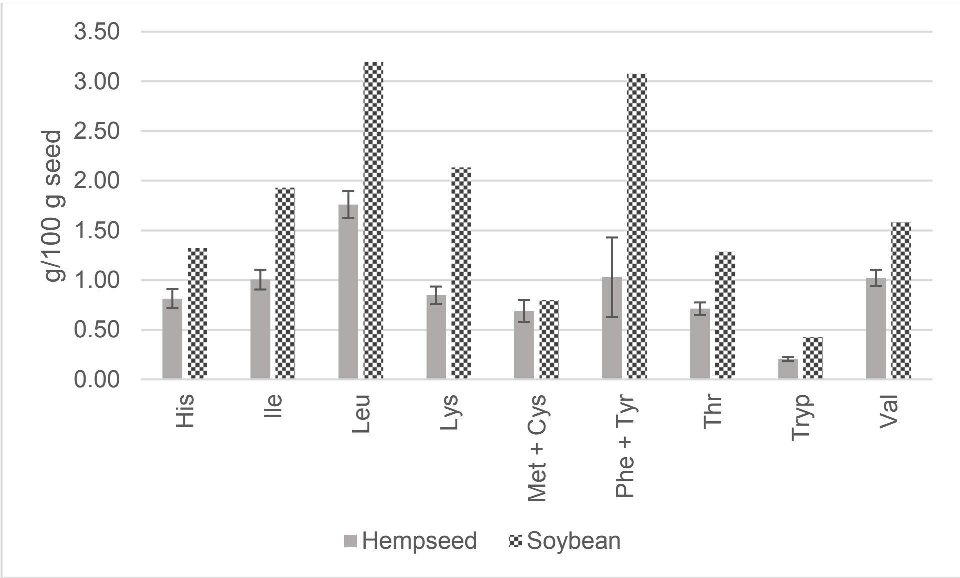

Figure 7. Content of essential amino acids (g/100g of seed) in whole hempseeds and soybean.

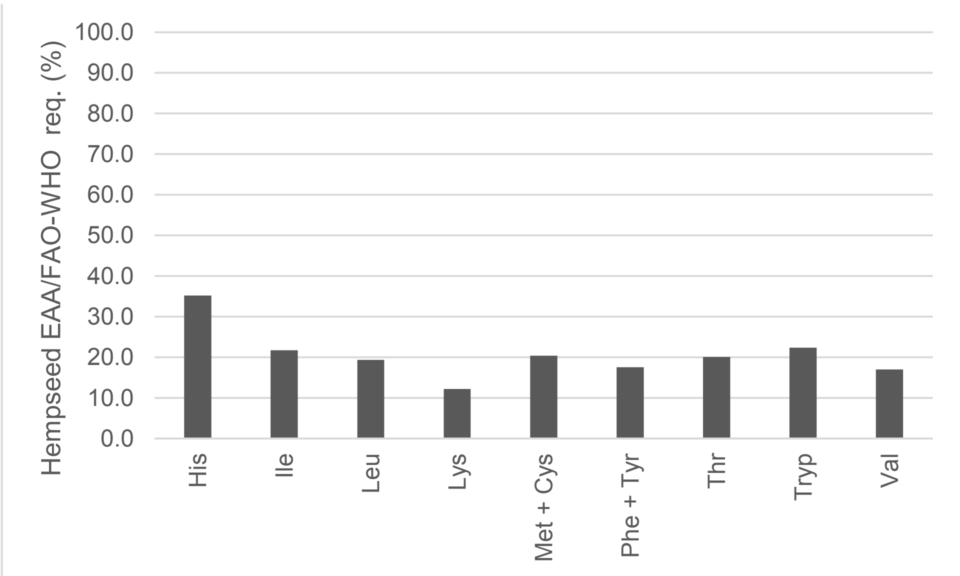

Figure 8. Contribution (%) to the FAO/WHO suggested essential amino acids requirements for a 70 kg adult of the habitual serving size (30 g) of whole hempseeds.

3.5.4 Mineral composition

Regarding the mineral content (mg/100g) of hempseeds, we can agree with other authors that they are a good source of minerals like potassium, magnesium, calcium, iron, manganese, zinc and coper [21,37]. Sodium was reported to be less than 5 mg/100g [45], nevertheless it was found as not detectable (<0.5 mg/100g) in all the hempseed samples herein analyzed. Other authors also reported high variability in the mineral content due to the plant variety [37]. Regarding hempseeds, the two macro elements potassium and magnesium were observed in higher concentrations. The average content of potassium was 799.84, which was reported to be the highest [4] or second highest mineral found in hempseeds [20,28,37] within the range of 250 – 2821 in the literature [37]. The average content (mg/100g) of magnesium and calcium was 370.26 and 130.57 resp., which is within the ranges of 237 – 694 and 90 - 955 reported in the literature for each mineral respectively [37]. The two micro elements manganese and copper were found in less quantity. The average content of manganese and copper were 8.41 and 1.09 resp., which is within the range of 4 - 15 and 0.5 - 2 reported in the literature for these two minerals [37]. The average content of iron was 12.78, within the range of 4 - 240 from the literature [37].

Comparing hempseeds to soybean, we can say that hempseeds are a richer source of micro elements (mg/100g) such as manganese, coper and zinc. Manganese, was found in hempseeds (8.41) but it was not present in soybean. The amount of coper and zinc were also higher in hempseeds than in soybean (Cu: 1.09 VS 0.67; Zn: 4.52 VS 2.67 resp.). On the contrary, calcium was found in amounts twice higher in soybean (294.17) than those in hempseeds (130.57).

A few studies regarding the content (mg/100g) of minerals on some whole hempseed varieties were found in the literature. For Fedora 17 v., the results of magnesium (298.57) in this experiment was consistent with reports in the literature with values of 268.21 and 410.9. Potassium (854.86) was higher than the values of 251.74 and 709.3 reported. Calcium (125.47) was within the values of 94.44 and 189.0 from the literature. Iron (6.98) was consistent to the given values of 6.45 and 9.80 from other studies. Zinc (4.47) was also close to the values of 4.84 and 6.69 found in the literature. Manganese (8.02) was higher than the 4.44 and 6.47 from the literature. Copper (1.21) was within the reported values of 0.50 and 2.76 [3,38]. In the case of Futura 75 v., our results showed a calcium content of 99.61, less than the 177.5 found in the literature [39]. Regarding Carmagnola v., the content of magnesium (321.14) in this study was a bit lower than the reported in the literature of 394.9. Potassium (1041.25) was higher than the value of 616.7 reported. Calcium (113.07) was lower than the value of 211.9 from the literature. Iron (16.58) was higher than the given value of 10.65 from other study. Zinc (3.05) was lower than the value of 9.71 found in the literature. Manganese (8.01) was close to the 9.71 from the literature. Copper (1.16) was also close to the reported value of 2.20 [45]. In the case of Felina 32 v., the results of magnesium (297.43) in this experiment was lower than the report of the literature of 367.1. Potassium (934.01) was higher than the value of 551.9 reported. Calcium (133.77) was lower than the value of 181.7 from the literature. Iron (14.31) was higher than the given value of 7.72 from other study. Zinc (3.08) was lower than the value of 7.02 found in the literature. Manganese (6.12) was slightly lower than the 7.41 from the literature. Copper concentration (1.00) was lower than the reported value of 2.67 [45]. For KC Dora v., the results of magnesium (499.90) in this experiment was higher than the report of the literature of 365.9. Potassium (744.45) was also higher than the value of 656.4 reported. Calcium (119.01) was lower than the value of 161.3 from the literature. Iron (5.85) was lower than the given value of 6.39 from other study. Zinc (5.58) was lower than the value of 7.11 found in the literature. Manganese (9.41) was higher than the value of 7.34 from the literature. Copper (1.10) was close to the reported value of 1.63 [38]. About Kompolti v., the amount of magnesium (470.11) in this study was higher than the report of the literature of 375.5. Potassium (652.69) was lower than the value of 713.6 reported. Calcium (128.7) was similar to the value of 137.3 from the literature. Iron (6.62) was slightly higher than the given value of 6.05 from other study. Zinc (5.10) was lower than the value of 7.11 found in the literature. Manganese (9.60) was higher than the 7.34 from the literature. Copper (1.20) was slightly lower than the reported value of 1.63 [45]. For Santhica 23 v., the concentration of magnesium (470.11) in this experiment was higher than the report of the literature of 329.62. Potassium (652.69) was lower than the value of 997.12 reported. Calcium (128.7) was similar to the value of 126.18 from the literature. Iron (6.62) was lower than the given value of 9.66 from other study. Zinc (5.10) was close to the value of 5.15 found in the literature. Manganese (9.60) was higher than the 7.79 from the literature. Copper (1.20) was close to the reported value of 1.05 [45]. Regarding Tiborszalassi v., the results of magnesium (322.80) in this experiment was lower than the reported by the literature of 410.6. Potassium (993.3) was much higher than the value of 415.1 reported. Calcium (107.83) was lower than the value of 172.0 from the literature. Iron (11.63) was higher than the given value of 9.70 from other study. Zinc (5.38) was lower than the value of 8.46 found in the literature. Manganese (12.48) was higher than the 8.46 from the literature. Copper (1.08) was slightly lower than the reported value of 1.76 [45]. All these variations may be due to factors like soil composition and fertilization [20,34,37].

When compared to the nutrient reference values (NRVs) of minerals established by the European Union [47] considering the average of the mineral content of the 29 whole hempseed varieties and a serving size of 30 g, hempseeds are nutritionally interesting for human consumption (Figure 9). Manganese was the mineral that could completely fulfill and exceed the NRV, and magnesium, coper and iron were able to cover more than the 25% of the NRV. In addition, whole hempseeds could be considered a sodium-free food because this mineral was less than 5 mg/100g [48].

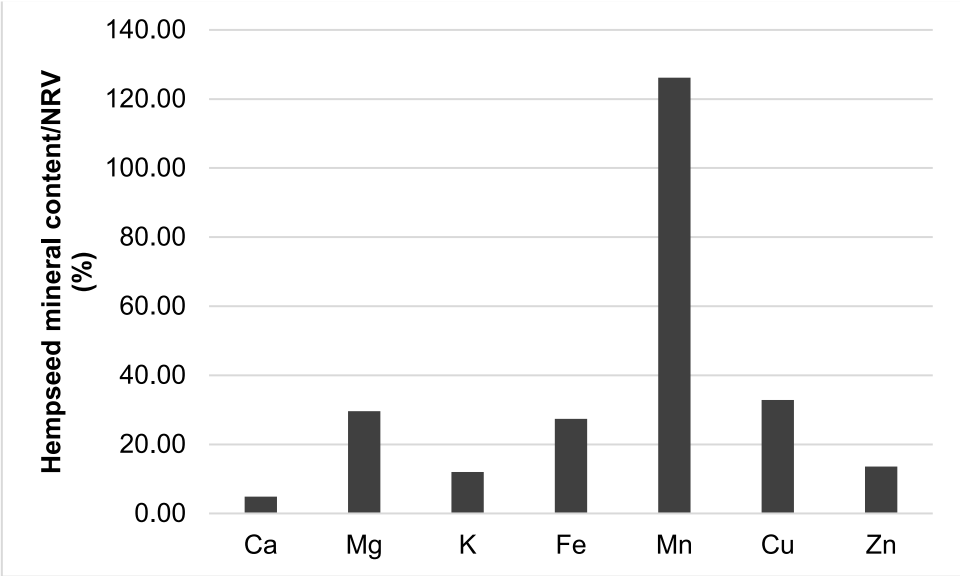

Figure 9. Contribution (%) to the nutrient reference values (NRV) of minerals of the habitual serving size (30 g) of whole hempseeds.

3.5.5 Cannabinoid content

Cannabinoids in industrial hempseeds had been reported to be very low [21,49] because their synthesis occurs in glandular trichomes that are not present in the seeds so this is why their content is associated to a contamination from vegetative materials [37]. The cannabinoids present in industrial hemp is under strong environmental influences [50]. Even though cannabinoids represent a very small quantity among all the industrial hempseed varieties, as reported in the literature [31], CBD concentrations were the highest between all the cannabinoids obtained in the 29 varieties with values that ranged from 8 to 92 µg/g. These results were higher than a previous study that reported CBD concentrations using the GC/MS technique in 77 commercial hempseeds for human consumption in a range of 0.32 to 25.55 µg/g (mean: 7.190 µg/g) [51]. Three other studies, which used the same technique and some of the hemp varieties herein analyzed, reported CBD values in a range of 1.125 to 2.039% [50], 1.0 to 1.27% [31] and 1.03 to 1.87% [52]. We can consider that these values were higher than ours because of the nature of the samples, the first one used the third upper part of the plant (including flowers, seeds and leaves) and the second and third study used the flowering tops. In addition, Helena v. had higher CBD content than Marina and Fedora 17 v. as reported in the literature [52].

THC leads the legislation of industrial hempseeds [36]. In accordance to the limit of 0.2%, this study found that 24 varieties did not contain any THC. From the five varieties that showed a small content of THC, four of them did not have the EU Registration. In fact, in the same studies discussed above, THC levels were also under the legal limit. The seventy-seven commercial hemp seeds showed lower THC concentrations that ranged from 0.06 to 5.91 µg/g (mean: 0.89 µg/g). The other studies, that used the top of the plant and the flowers, reported ranges of 0.137 to 0.581% [50], 0.05 to 0.07% [31] and 0.08 to 0.10% [52] respectively. A comparison between

Futura 75 v. seeds could be done with a study on this variety that reported a CBD content of 561 μg/g and a THC of 212 μg/g, but the technique used was HPLC-MS [33]. These values were higher than the ones we found in the same variety, being 9 and 0 μg/g for CBD and THC respectively.

3.6 Conclusions

A complete and comprehensive composition study of 29 varieties of whole industrial hempseeds is presented. They contain interesting amounts of fat and protein. Hempseed varieties analyzed in this work contain high amounts of PUFA and the primary fatty acids detected were linoleic acid and α-linolenic acid. Moreover, the amino acid profile of hempseeds constitutes a good source of essential amino acids and it was found that arginine and glutamic acid were the most abundant amino acids. Regarding the cannabinoid content, CBD and THC were present at very low values. Interestingly, Tisza v. was balanced for crude protein, fat and also amino acid content. However, more studies are needed to quantify the presence of antinutritional compounds, phenolic compounds and bioactive peptides. To conclude, the nutritional composition of hempseeds with hull makes them suitable to be added into humans or animals' diet as a highly beneficial novel ingredient. Moreover, knowing that the chemical composition and the presence of bioactive compounds of cultivated plants is strongly influenced by the environment in which they grow, we recommend to perform similar studies in other countries in order to have a wider approach regarding this topic.

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Chapter 4.

Effect of Dietary Hemp Cake Inclusion on the In Vivo and Post Mortem Performances of Holstein Veal Calves

4.1 Abstract

Fifty-two male Holstein veal calves were divided into two homogeneous groups receiving two isoenergetic and isonitrogenous concentrates without (CTR group) or with 3% of hemp cake (HC group). The trial lasted for 171 days. All the calves were weighed five times during the trial. At slaughtering, carcasses were weighed and measured. Meat quality was determined on the Longissimus dorsi muscle. Average daily gain in the first period of the experiment (0–80 d) and dressing percentage and rump width of the carcasses were higher in HC group. Cooking weight losses and shear force were higher in the meat of the HC group while color parameters were similar in the two experimental groups. Unexpectedly, the alpha-linolenic acid content of meat was lower in the HC group. In conclusion, hemp cake can be considered an interesting ingredient in the concentrate used for the production of veal calves, but further studies will be needed to determine a suitable dosage in order to improve the nutritional quality of meat (i.e., the n-3 fatty acids content) without negative effects on physical characteristics.

4.2 Introduction

Hemp (*Cannabis sativa* L.) is a multifunctional crop [1]. Around 60% of the total worldwide hemp production takes place in Europe [2]. Hemp is grown for various applications, mostly to obtain fiber for making light-weight papers, insulation material and biocomposites [3]. Hemp seeds are used predominantly in human [4] and animal nutrition [5–9] and they can be cold-pressed to obtain oil and hemp cake. Both co-products are used in animal feed [3,8]. Hemp cake is an optimal source of protein (on average 34.3 ± 2.1% on DM basis) and energy, considering the high percentage of residual oil (12.7 ± 2.8% on DM basis) [6,10]. In addition, the oil shows a good fatty acid profile, with a high content of PUFA (on average 75% of total fatty acids), especially linoleic acid (LA; 18:2 n-6) and linolenic acid (ALA, 18:3 n-3) [6].

Essential fatty acids (FA) play an important role in human health and a valuable source of these nutrients is meat. Unfortunately, meat consumption seems to be associated with two of the major chronic diseases in the Western world: cardiovascular disease and colon cancer, probably because of the saturated fatty acid (SFA) content [11]. In fact, the fatty acid profile of meat is primarily monounsaturated fatty acids (MUFA) (45–50%) and SFA (45–55%), and very low amounts of PUFA (10%) [12,13]. In order to reduce the risk of cardiovascular diseases, guidelines for decreasing total FA intake, and replacing SFA with PUFA, especially those of the n-3 series, has been recommended by the World Health Organization [14]. This is why meat consumers not only consider a low level of fat content mandatory, but also see the fatty acid composition of any meat as an important point of consideration [15].

To guarantee the production of healthier meat, various investigations have been made over the last decade. One method that has increased in popularity is manipulating the fatty acid profile, specifically increasing the n-3 fatty acid content of beef to increase its appeal in a healthy diet [12]. Knowing that the three major factors that influence the FA composition of beef are the age, breed and diet of the animal [11], many researchers have chosen to work on the animal’s diet because fatty acid metabolism in the rumen depends primarily on this [16]. Despite extensive ruminal modification, diets high in specific unsaturated fatty acids can increase the concentration of FA in deposited fats [17]. These diets include the dietary supplementation of oilseeds [12] that can reduce the ruminal modification of FAs and may therefore increase the resemblance between dietary and tissue FAs [17]. Supplementation of the diet with PUFA-rich oilseeds such as hemp has led to increases in conjugated linoleic acid isomer (CLA) levels [16] and some beneficial effect of hemp supplementation on the fatty acid composition has been shown in chicken [18] and duck meat [19]. Moreover, one study reported that hemp seeds can favorably alter carcass fat by increasing levels of CLA and n-3 fatty acids in beef without negatively affecting the animal performance [17]. No other studies have been carried out on meat with hemp to increase knowledge regarding this source of PUFAs.

The use of hemp in healthy meat production may also have an advantage, as it is rich in an antioxidant called tocopherol [20]. Producing beef with meaningfully enhanced concentrations of biohydrogenation products can alter meat quality because PUFAs are more susceptible to oxidation and can cause surface discoloration [12]. This is an issue, as meat color is most often used as an indicator of product freshness and quality by consumers [12]. Therefore, the presence of tocopherol may also help to keep meat color parameters unaltered.

Since altering the nutritive value of meat has become the focus of a number of producers [12], new sources of oilseeds need to be tested for this purpose. The objective of this study was to evaluate dietary hemp cake inclusion in Holstein veal calves on in vivo and post mortem performance, with particular attention on meat quality and fatty acid profile.

4.3 Materials and Methods

4.3.1 Ethics Statement

All experimental procedures were carried out according to Italian law on animal care (Legislative Decree No. 26 of 14 March 2014) and approved by the ethical committee at the University of Padova (approval number 38/2022).

4.3.2 Animals and Diet

The experiment was conducted on a farm located in the province of Treviso, in the north-east of Italy. A total of 52 Holstein male calves with 25 ± 13 d of age were chosen. The calves were randomly assigned, based on their initial body weight (BW), into two homogeneous groups called control (CTR) and hemp (HC). They were housed in one room divided into 10 pens (5 per group). Each pen housed 4 to 6 calves, with a space allowance of 1.8m²/calf. Moving from pen 1 to pen 5, the average weight or size of the calves increased but remained homogeneous inside the pen in order to avoid hierarchical or aggressive behavior. The pens had wooden slatted floors. There were 3 feeders per group, every two pens sharing one and the last pen having an individual feeder. Environmental temperature in the house was controlled and maintained by an extractor fan system at 22 C°. The experimental period lasted for 171 days.

The calves from the two experimental groups were fed the same commercial milk replacers during the whole trial. Two milk replacers that met the calves' nutrient requirements during the fattening period were used. The first milk replacer (Denkaveal Start, Denkavit Italiana S.r.l, Brescia, Italy) was used during the adaptation period (24 days) and then in the first 7 days of the experimental period. During the next 29 days, the first milk replacer was mixed in a 50:50 ratio with the second milk replacer (Sharmel Unico Light S, Frabes S.p.A., Brescia, Italy). In the following part of the experiment (135 days), calves received only the second replacer. The daily dose of milk was delivered in two equal meals at 06:30 and 18:30. The daily amount of milk powder and its concentration in the liquid diet increased throughout the trial from 830 to 2120 g per calf per day for the two experimental groups.

Two isoenergetic and isonitrogenous concentrates with conventional protein sources and with 3% of hemp cake were formulated, respectively, for the control group (CTR:26 animals; 5 pens) and hemp cake group (HC: 26 animals; 5 pens). The hemp (*Cannabis sativa* L.) used in the trial was Futura 75 variety, cultivated in Ferrara (Italy). The oil was extracted from seeds using a cold process by the company Vergavara Lab (Rossano Veneto, Vicenza, Italy). The residual hemp cake was used by the feed company Italfiocchi Monfort S.r.l. (Castelfranco Veneto, Treviso, Italy) to produce the HC concentrates. The chemical composition of hemp cake is reported in Table 1.

Table 1. Chemical composition (% on DM), iron content (mg/kg) and fatty acid profile (g/100g of total FA) of the hemp cake.

Hemp cake	
Chemical composition	
DM	92.40
Crude Protein	28.17
Lipids	8.70
Ash	6.19
NDF	50.91
ADF	37.38
ADL	11.54
AIA	0.72
Iron	168.80
Fatty acids	
C14:0	0.07
C16:0	8.28
C18:0	3.04
C18:1n-9	15.79
C18:2n-6	56.20
C18:3n-3	12.82
Total SFA[1]	13.33
Total MUFA[2]	17.46
Total PUFA[3]	69.21
n-6/n-3	4.4

[1]SFA, saturated fatty acids; [2]MUFA, monounsaturated fatty acids; [3]PUFA, polyunsaturated fatty acids.

During the adaptation period (24 days) all the animals received the control diet through a concentrate 1 (Denkaveal Avance PI Integrato Fiocco +P DP, Fanin S.p.A., Vicenza, Italy). The experimental period was divided into two phases. In the first phase (61 days), the CTR group was fed with concentrate 1 and the HC group received concentrate 2 (Avance Mix Fiocco Fiber 5% Canapa, Italfiocchi Monfort S.r.l., Treviso, Italy). In the second phase (110 days), the CTR group was fed with concentrate 3 (Avance Mix Fiocco Omega Fiber 5%, Italfiocchi Monfort S.r.l., Treviso, Italy) and the HC group with concentrate 4 (Avance Mix Fiocco Omega Fiber 5% Canapa, Italfiocchi Monfort S.r.l., Treviso, Italy). The inclusion of the hemp meal was made by substituting 6 and 4.5% of lupin seeds in the first and second phase respectively. The concentrates were distributed ad libitum. During the trial the amount of concentrate increased on average from 943 to 3922 g/d. The DM intakes of each group were recorded daily. For each pen, the total DM intake (milk re-placer and concentrate) was calculated.

The chemical composition and fatty acids profile of milk replacers and concentrates are reported in Table 2 and Table 3 respectively.

Drinking water was available ad libitum.

Table 2. Chemical composition (% on DM) and iron content (mg/kg) of milk replacers (MR) and concentrates (C).

			CTR		HC	
Item	**MR1[1]**	**MR2[2]**	**C1[3]**	**C3[4]**	**C2[5]**	**C4[6]**
Dry matter	94.05	93.98	89.95	90.69	90.04	90.48
Crude protein	24.27	18.71	13.12	13.02	13.87	13.51
Lipids	18.31	15.77	3.34	2.36	2.25	2.37
Ash	8.02	6.72	4.32	4.11	4.65	4.28
Starch	nd	nd	38.62	40.70	41.95	39.62
NDF	nd	nd	18.60	24.09	22.92	27.22
ADF	nd	nd	7.99	10.61	10.45	13.20
Iron	19.26	16.77	63.34	64.25	40.72	68.99

[1]Ingredients of Milk replacer 1: proteins of whey-milk powder, whey-powder, de-lactosed whey powder, animal fats (pork and bovine), wheat protein, vegetal oils (coconut, soy), wheat meal, protein of extruded peas, protein concentrate of soybean seed, calcium carbonate, monopotassium phosphate, magnesium oxide, fatty acids of tall oil, 1,2–propanediol. Vitamin and mineral content (per kg): 12,450 IU of vitamin A; 3,900 IU of vitamin D3; 80 IU of vitamin E; 8 mg of Fe; 0.5 mg of I; 10 mg of Cu; 30 mg of Mn; 40 mg of Zn; 0.1 mg of Se.[2]Ingredients of Milk replacer 2: whey-powder, animal fats (pork and bovine), delactosed whey powder, wheat protein, wheat meal, vegetal oils (coconut, soy), protein concentrate of soybean seed, protein of extruded peas, dextrose, calcium carbonate, monopotassium phosphate, magnesium oxide, 1,2–propanediol. Vitamin and mineral content (per kg): 12,500 IU of vitamin A; 2,000 UI of vitamin D3; 80 IU of vitamin E; 0.5 mg of I; 3 mg of Cu; 10 mg of Mn; 80 mg of Zn; 0.1 mg of Se. [3,4]Ingredients of Concentrates 1 and 3: corn flakes, corn grain, wheat straw, barley flakes, white lupin flakes, barley seed, white lupin seed, pea flakes, corn gluten meal, calcium carbonate, sodium chloride and vitamins.[5,6]Ingredients of Concentrate 2 and 4: corn flakes, corn grain, wheat straw, barley flakes, white lupin flakes, barley seed, white lupin, pea flakes, hempseed meal, corn gluten meal, calcium carbonate, sodium chloride and vitamins. [3,5]Vitamin and mineral content (per kg): 37.21 mg of vitamin E; 0.47 mg of Co; 0.58 mg of I; 23.25 mg of Mn; 0.28 mg of Se.[4,6]Vitamin and mineral content (per kg): 39.90 mg of vitamin E; 0.50 mg of Co; 0.60 mg of I; 24.93 mg of Mn; 0.30 mg of Se.

Table 3. Fatty acid profile (g/100g of FAME[1]) of the concentrates.

	CTR		HC	
Fatty acid	**Concentrate 1**	**Concentrate 3**	**Concentrate 2**	**Concentrate 4**
C14:0	0.38	0.22	0.30	0.40
C16:0	21.08	15.50	14.44	16.22
C18:0	3.34	3.34	2.26	2.39
C20:0	0.86	0.62	0.67	0.74
C22:0	0.50	0.77	0.37	0.41
C24:0	0.45	0.41	0.33	0.38
Total SFA	27.69	21.29	18.78	21.11
C18:1n9	30.78	30.85	26.29	27.60
C20:1n9	0.48	0.42	0.47	1.00
C22:1n9	0.19	0.13	0.10	0.12
Total MUFA	32.35	32.28	27.68	28.99
C18:2n6	38.05	43.90	50.39	46.87
C18:3n6	0.04	0.03	0.19	0.09
C18:3n3	1.68	2.36	2.81	2.74
Total PUFA	39.97	46.42	53.54	49.89
Total n-6	38.19	44.01	50.69	47.09
Total n-3	1.77	2.41	2.85	2.81
n-6/n-3	21.5	18.2	17.8	16.8

[1]FAME, fatty acid methyl esters; [2]SFA, saturated fatty acids; [3]MUFA, monounsaturated fatty acids; [4]PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

4.3.3 Animal Monitoring

Upon arrival, calves were vaccinated, treated for external and internal parasites and, checked by a veterinarian to verify their health status. Also, all the animals were supplemented orally with 1g Fe/day (Ferro tonic, Daily Manufacturing, USA) from day 11 to day 20 of the trial.

During the experiment all calves were individually weighed five times (at day 1, 38, 80, 122 and 171) in order to assess the average daily gain (ADG). Dry matter efficiency was estimated as daily gain/DM intake ratio.

The health status of the calves was monitored through blood analysis at the beginning, middle and end of the experimental period. Blood samples were taken from all the calves by jugular vein puncture before the morning meal. Heparinized vacutainer tubes (FL MEDICAL SRL, Padova, Italy) were used for assessing plasma hemoglobin and iron concentration according to procedures PDP ACC 075 2022 Rev.1 and PDP ACC 043 2018 Rev.1 respectively of Istituto Zooprofilattico Sperimentale delle Venezie (Legnaro, Padova, Italy).

At the end of the trial, 51 calves were slaughtered in an authorized, commercial slaughterhouse (Bencarni S.p.a., Nogarole Rocca, Verona, Italy) following the recommendations of the European Council regarding the protection of animals at the time of killing. Around 30 minutes after slaughter, the hot carcass weight was measured and the individual dressing percentage was calculated. The thigh length, rump width and pH were measured on each hot carcass. Then, the carcasses were aged in a controlled room for 7 days at a temperature of 0°-4°C.

4.3.4 Chemical and Technological Analyses

Samples of hemp cake, milk replacers and concentrates were collected at the beginning and at the end of the experiment and analyzed for dry matter, protein, lipids and ash according to the association of official analytical collaboration [21]. Neutral detergent fiber (NDF), inclusive of residual ash, was determined with α-amylase using the Ankom220 Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Acid detergent fiber (ADF), inclusive of residual ash, was determined sequentially after NDF determination [22]. Starch content was determined after hydrolysis to glucose by liquid chromatography [23].

To quantify the iron content, a mineralization was performed using a Start D microwave digestion system (Milestone Srl., Sorisole, BG, Italy). The samples were digested with 7 mL of HNO_3 super pure and 2 mL of H_2O_2 30% by bringing them up to 200°C in 15 min and remaining at that temperature for a further 15 min. The digested samples were diluted with deionized water into 25 mL volumetric flasks. The concentration of iron was measured by ICP-MASS Spectrometry (EPA 6020A 2007).

To evaluate meat quality characteristics, 7 days after slaughter, 24 samples of Longissimus dorsi muscle (12 for each experimental group) were taken from the fifth–sixth rib. The sample was vacuum-packaged and stored at 4°C in a chilling room for 6 days. After this aging period, the meat samples were frozen and kept at -20°C until analysis.

Meat chemical analyses considered moisture, intramuscular fat (IMF), protein content and iron concentration (see above). Technological analyses considered pH, color, cooking losses and shear force. The pH was measured by a portable pH-meter provided with a 5050T electrode (Hach Lange S.r.l., Milan, Italy). Meat color was measured with a CM-600d spectrophotometer (Konica Minolta Inc., Tokyo, Japan) on samples following the AMSA method [24]. Samples of meat were measured by scanning 3 different spots and color data were expressed according to the CIE L*, a* and b* system. The instrument was calibrated with a white standard plate before measurements. Weight cooking losses were determined on 2 cm thick steaks heated in a water bath at 75°C for 60 min and cooled in running tap water for 15 min. After cooking and before opening the bags, each sample was tempered at room temperature to drain liquid.

The cooking losses were calculated using the formula:

(weight of raw meat - weight of cooked meat)/weight of raw meat x 100.

The instrumental measurement of meat tenderness was carried out using a Lloyd Instrument LS5 shear force meter (AMETEK Inc., Thurmaston, LE, UK) on five cylindrical core samples of cooked meat of 1.4 cm in diameter. The measurement was recorded and calculated with the instrument software Newygen Plus 3 as the peak yield force in N, required to shear, at a 250 mm/min crosshead speed, perpendicularly to the direction of the fibers on five cylindrical cross-section (4 x 8 x 1 cm dimension) replicates from each sample [25].

4.3.5 Fatty acid profile of feed and meat

The fatty acid profile of samples (feed and meat) was determined by a preliminary extraction of fat using an accelerated solvent extraction (ASE 200, Dionex Corp., Sunnyvale, CA, USA) with petroleum ether. The GC with flame-ionization detector (Agilent Technologies Inc., Shanghai, China) had a temperature of 300 °C and was equipped with two columns in series and with a modulator (Agilent G3486 A CFT), an automatic sampler (Agilent 7693) and specific machine software (Agilent Chem Station) were used to determine the concentration of the single fatty acids. This instrument was chosen because the double column allows for separating and identifying each FA on a 2-dimensional basis [26]. The first column was a 75 m x 180 μm (internal diameter) x 0.14 μm film thickness column (23,348U, Supelco, Bellefonte, PA, USA), the second was a 3.8 m x 250 μm (internal diameter) x 0.25 μm film thickness column (J&W 19091-S431, Agilent Technologies). The first and the second column used H_2 as carrier gas at a flow rate of 0.25 and 20 mL/min, respectively.

The concentration of each fatty acid was expressed as g/100 g, considering 100 g as the total of areas of all FAME identified.

4.3.6 Statistical Analysis

The normal distribution of all the variables included in the dataset were tested and then submitted to an ANOVA within PROC GLM (SAS Inst. Inc., Cary, NC, USA). For performance parameters, data were analyzed using a nested design in which each calf was an experimental unit, and the effect of the pen was considered.

$$Y_{ij} = \mu + D_i + D_i (\beta_j) + \varepsilon_{ij}$$

where Y_{ij} are the observations, μ the overall mean, D_i the effect of the diet (i = 2), β_j the effect of the pen and ε_{ij} is the random residual.

For blood parameters, a mixed model using the period (3 periods) as an effect was used. Data of meat parameters were submitted to a completely randomized design. For all the variables, the comparisons between LS means were performed using the Tukey test and differences were considered significant at $p < 0.05$.

4.4 Results

4.4.1 Animal Performance

One calf of the CTR group died during the trial. The cause of death was due to a respiratory problem. For this reason, this animal was discarded from the data set.

The use of hemp cake in veal calves' diet did not affect ($p > 0.05$) the final body weight or the average daily gain of the whole trial. Similarly, DM intake (milk and concentrate) and feed efficiency for both groups were not affected (Table 4).

The "pen" effect was significant for all the variables reported in Table 1, but this is an expected result considering that the distribution of calves in the five pens, within each experimental group, was based on the initial BW, as reported above.

Table 4. Effect of dietary hemp cake inclusion on in-vivo performance of veal calves.

	Diet[1]			p-value	
Item	**CTR**	**HC**	**SEM[3]**	**Diet**	**Pen**
Initial BW, kg	59.7	59.7	0.75	0.883	<0.001
Final BW, kg	326.2	323.1	5.64	0.722	<0.001
ADG[2], kg/d	1.507	1.489	29.23	0.710	<0.001
Feed consumption					
Milk replacer, kg DM/d	1.241	1.241	--	--	--
Concentrate, kg DM/d	2.530	2.601	0.20	0.370	0.014[4]
Feed conversion ratio	2.502	2.533	0.04	0.332	--

[1]Dietary treatments: CTR group: fed concentrate without hemp inclusion; HC group: fed concentrate with 3% hemp cake. [2]ADG=average daily gain. [3]SEM=standard error of least square means. [4]Instead of pen, feeders were used as block for concentrate consumption

Considering the pattern of growth, the maximum value of ADG was observed from day 81 to 122 of the trial for both experimental groups (1830 and 1719 g/d for CTR and HC groups, respectively; $p > 0.05$). Only during the first phase were the differences of ADG between CTR and HC group statistically significant, showing higher values for the HC group (1017 vs. 1141 g/d, respectively, from day 1 to 38 and 1417 vs. 1533 g/d from day 39 to 80; $p < 0.05$) (Figure 1).

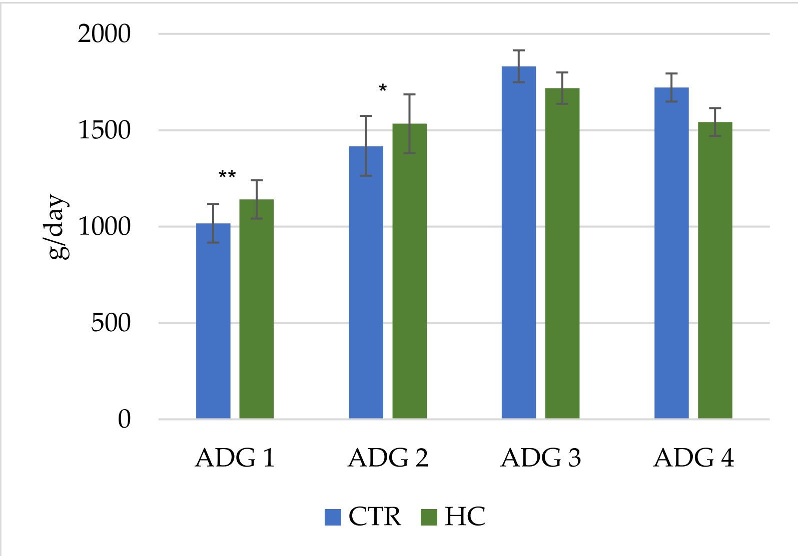
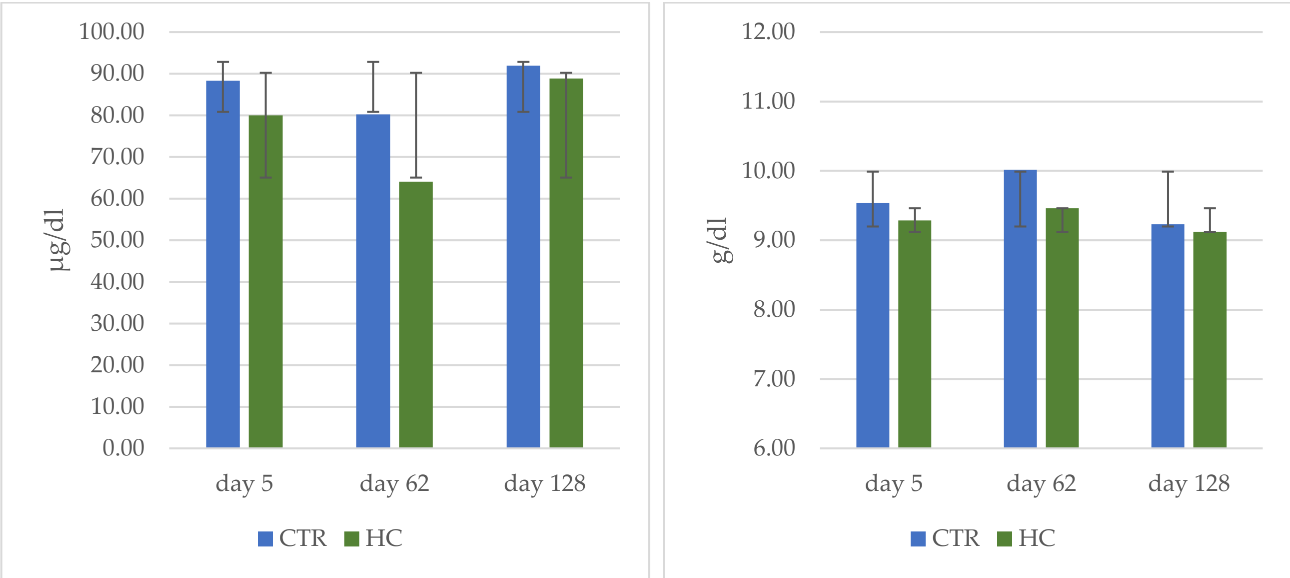


Figure 1. Least square means of average daily gain for control (CTR; blue bars) and hemp cake (HC; green bars) groups of veal calves during the different phases of the experiment (ADG1; 1-38 days, ADG2; 39-80 days, ADG3; 81-122 days, ADG4; 123-171 days). Differences between treatments within phase have been reported when significant: (*) $p < 0.05$, (**) $p < 0.01$.

4.4.2 Animal Health Status

The incidence of respiratory problems was very low in both groups (19.2 vs. 11.1% in CTR e HC group, resp.) and few cases of gastrointestinal problems were observed in the HC group (7.41%). In the blood samples taken at 5, 62 and 128 days of the experiment, the iron concentration (Figure 2a) was similar in the two groups (on average 84.95 µg/dL). The hemoglobin concentration (Figure 2b) was lower and more constant during the fattening period in the HC group in comparison to CTR group (9.64 vs. 9.33 g/dL; $p < 0.05$).



(a) **(b)**

Figure 2. Health status parameters for CTR (control group) and HC (Hemp group) taken in three different periods: Initial, at the beginning of the trial; Intermediate, in the middle of the trial and Final, at the end of the trial. (a) Iron values (b) Hemoglobin values.

4.4.3 Meat Characteristics

Even though the carcass weight was similar for both groups (162.8 vs. 164.6 kg for CTR and HC group, resp.; $p > 0.10$), the dressing percentage was higher (49.9 vs. 51.5%; $p < 0.001$) in the HC group. In addition, the conformation of carcasses was better in the HC group, considering, in particular, the rump width (37.20 vs. 38.38 cm; $p > 0.05$). The pH of carcasses was not affected ($p > 0.05$) by the hemp cake inclusion.

The color parameters (L, a, b, H, C,) of meat obtained by the Minolta spectrophotometer were very similar between experimental groups (Table 5). The lightness of meat in the HC group tended ($p = 0.10$) to be higher than that of the CTR group. The weight cooking losses and shear force showed significantly higher values in the HC group (29.49 vs. 31.13%; $p < 0.05$ and 25.79 vs. 36.19 N; $p < 0.001$ respectively).

Regarding the chemical composition of the Longissimus dorsi muscle, no differences ($p > 0.05$) were observed for water, intramuscular fat (IMF), protein and iron content.

Table 5. Effect of dietary hemp cake supplementation on carcass traits and physicochemical properties of *Longissimus dorsi* muscle at 7 days of storage of Holstein veal calves.

	Diet			
Item	**CTR**	**HC**	**SEM**	**p-value**
Carcass parameters				
Carcass weight, kg	162.78	164.55	2.91	0.7644
Dressing percentage, % BW	49.91^{b}	51.47^{a}	0.24	0.0007
Thigh length, cm	67.92	67.81	0.64	0.8996
Rump width, cm	37.20^{b}	38.38^{a}	0.44	0.0496
pH	5.76	5.69	0.02	0.0663
Meat characteristics				
Lightness (L*)	45.83	47.34	0.46	0.1010
Redness (a*)	7.84	7.77	0.23	0.8834
Yellowness (b*)	14.95	14.90	0.16	0.8775
Hue angle (H*)	62.47	62.62	0.59	0.9060
Chroma (C*)	16.90	16.84	0.22	0.8964
Weight cooking losses (%)	29.49^{b}	31.13^{a}	0.38	0.0298
Shear force (N)	25.79^{B}	36.18^{A}	1.50	<0.0001
Water content (g/100g muscle)	74.05	74.31	0.22	0.5847
IMF content (g/100g muscle)	3.75	3.67	0.27	0.8911
Protein content (g/100g muscle)	22.78	22.59	0.14	0.5060
Iron (mg/kg muscle)	2.83	2.95	0.09	0.5128

Dietary treatments: CTR group: fed concentrate without hemp supplementation; HC group: fed concentrate with hemp supplementation. IMF=Intramuscular Fat. ^{a,b} Mean values with different letters in superscript within rows indicate significant differences ($p<0.05$); ^{A,B} Mean values with different letters in superscript within rows indicate significant differences ($p<0.01$); SEM=standard error of least square means.

4.4.4 Fatty Acid Profile of Meat

For both experimental groups, the most represented SFA in meat was palmitic acid (C16:0, on average 26.5% of the total FA) followed by stearic acid (C18:0, on average 12.3% on the total FA) (Table 6). The most abundant unsaturated fatty acid proved to be oleic acid (C18:1 n9), which was higher in the HC group as compared to the CTR group (38.9 vs. 40.3% of total fatty acids; $p < 0.05$). The inclusion of hemp cake in concentrate caused a decrease in the n-3 fatty acids, mainly represented by alpha linolenic acid (ALA, C18:3 n3, 0.45 vs. 0.36% of the total FA; $p < 0.05$). The n-6:n-3 ratio, considered an important nutritional index, was higher in the HC group (16.8 vs. 19.9 for CTR and HC group, respectively; $p < 0.05$).

Table 6. Effect of dietary hemp cake supplementation on fatty acid profile (g/100g of total FA) of total lipids in the *Longissimus dorsi* muscle of Holstein veal calves.

	Diet			
Fatty acid	**CTR**	**HC**	**SEM**	**p-value**
C12:0	0.46	0.45	0.03	0.8632
C14:0	5.05	4.91	0.12	0.5377
C16:0	26.33	26.58	0.29	0.6835
C18:0	12.41	12.11	0.25	0.5490
Total SFA	45.48	45.07	0.39	0.6094
C14:1	0.67	0.72	0.03	0.3907
C16:1n7	3.35	3.25	0.07	0.4394
C18:1 n9	38.94^{b}	40.27^{a}	0.34	0.0492
C18:1 t11	3.06^{a}	2.71^{b}	0.09	0.0466
Total MUFA	46.81	47.55	0.31	0.2403
C18:2 n6	7.19	7.01	0.19	0.6608
C18:2 CLA	0.08	0.01	0.02	0.1243
C18:3 n3	0.45^{a}	0.36^{b}	0.02	0.0299
Total PUFA	7.71	7.38	0.21	0.4345
n6:n3 ratio	16.77^{b}	19.85^{a}	0.66	0.0157

The concentration of fatty acids was expressed as g/100g, considering 100g the sum of the areas of all identified FAMEs. Dietary treatments: CTR group: fed concentrate without hemp supplementation; HC group: fed concentrate with hemp cake supplementation. IMF=Intramuscular Fat. ^{a,b} Mean values with different letters in superscript within rows indicate significant differences ($p<0.05$); SEM=standard error of least square means. SFA=Saturated fatty acids; MUFA=Monounsaturated fatty acids; PUFA=Polyunsaturated fatty acids.

4.5 Discussion

To our knowledge, no scientific papers have been published on the use of hemp cake in the diet of veal calves.

4.5.1 In Vivo Performance

The results of this experiment showed a good growth and fattening during the whole (171 d) trial for Holstein calves fed concentrate with the inclusion of hemp cake. The replacement of traditional protein and energy sources with the product obtained as residual material of the cold hemp oil extraction allows high in vivo performance to be obtained. The calves receiving hemp cake in the concentrate reached the slaughter weight at the same time as the animals fed the control diet or conventional diets [27].

In the first phase of the fattening period (1–80 day), the daily gain of calves receiving hemp cake was significantly higher than that of the control group. It could therefore be hypothesized that hemp cake is more efficient in sustaining growth in the first phase of life when protein daily gain begins and prevails over fat gain. This hypothesis is supported by the recent results of hemp seed protein in human nutrition reported by Farinon et al. [4].

The authors recognized that whole hemp seed can be considered not only a rich-protein source (25.6% of crude protein on DM), higher than or similar to other protein-rich products (i.e., flaxseed, 20.9 and lupin seed, 30.5% of crude protein on DM) [28] but also a good source of essential amino acids. The amino acids profile of hemp seed (Finola variety) is similar to those of casein in milk except for lysine, which is the first limiting amino acid of hemp seed [4,29]. In this experiment, the 6% of hemp cake was included in the concentrate in replacement of white lupin seed in the first phase. As reported by Mattila et al., the lysine content of hemp seed is lower than that of white lupin seed (3.30 vs. 5.80 g/100 g of protein), however, on the contrary, methionine level is very high in hemp cake (2.19 vs. 0.80 g/100 g protein) [28]. In the first period, veal calves ingest a high quantity of milk replacer, based on whey derivatives, which could have satisfied the requirements of lysine for growth. Therefore, it is possible to hypothesize that the hemp cake inclusion could be useful, in this phase, for providing a suitable quantity of the secondary limiting amino acid, that is, methionine.

In the second phase of the fattening period, the values of daily gains of calves were similar in the two experimental groups, probably owing to a compensatory growth of subjects receiving the concentrate without hemp cake. In addition, the percentage of hemp cake inclusion in the concentrate of the HC group during the second period was lower (4.5% instead of 6%). Also, the amino acid requirements change in relation to the age and weight of calves following the modifications of the chemical composition of daily gain.

Considering the whole experiment, the DM intake of concentrate is similar for both groups. This indicates that the palatability of the concentrate containing both 6% and 4.5% of hemp cake was high for veal calves. Hessle et al. found an increase in total DM intake when a high quantity of hemp cake (1 kg/d) is included in place of soybean meal in the diet of dairy calves (from 96 to 250 kg BW) fed mixed rations based on grass/clover silage and rolled barley [30].

No differences in feed conversion ratio between the two experimental groups were observed in this experiment. Feed conversion ratio was similar to that reported recently by Van Gastelen et al. in an experiment with Holstein-Friesian calves fed different solid feed mixtures, but considering a short experimental period (from 13 to 17 weeks of age) [31].

Regardless of the feeding treatment, the health status of the calves was considered optimal as shown by the normal levels of hemoglobin and iron. Special consideration for these two parameters is given in this type of breeding because of the regulations that state a minimum hemoglobin level of 7.25 g/dl [32]. The hemp supplementation significantly decreased the calves' hemoglobin in blood, but they were never below the recommended level. The iron content in blood was not affected by the treatment, which means that this level of hemp supplementation can be considered safe for the animal's welfare. In conclusion, the iron content provided by the hemp cake in the concentrate allows a good state of health and welfare of the calves without compromising the color of the meat, which must be pale as shown below.

4.5.2 Post Mortem Performance

Hemp cake inclusion did not affect the carcass weight, but the dressing percentage was higher for the HC group. Dressing percentage values (49.91 and 51.47% from the CTR and HM group, resp.) in this trial are lower than the average of 54.8% calculated from previous studies in Holstein calves slaughtered at weights between 210 to 280 kg [27,33–35]. Although the supplementation (full-fat hemp seed vs. hemp cake) and the category of animals (steers vs. calves) are different, these results are consistent with a previous study of Gibbs et al. in which the effects of full-fat hemp seed were not significant in steers fed with a barley-based finishing diet [17]. However, rump width was significantly improved with hemp inclusion. This parameter measures the carcass in terms of size and describes the animal growth better than conventional methods of weighing [33].

Regarding the instrumental color variables, the results obtained in this experiment are in agreement with those reported by Brugiapaglia et al. in white veal calves (L* 53.37, a* 6.91, b* 15.15, C* 16.76, H* 65.75) [36], showing a very light pale color, as it should be, due to high L* values together with low a* and high b* values. For parameters such as redness (a*) and color vividness (C*), the values 12 and 16, respectively, are considered as thresholds for visual discoloration in beef. In this case, as we are dealing with white veal meat, it was normal to find values below these two suggested for red beef. Also, as the veal industry depends strongly on lean color, and whiter graded carcasses command greater value [34], and knowing that hemp is a source of PUFA, its addition in the diet could have made the meat more susceptible to oxidation, thus causing surface discoloration [12]. However, according to our results, all these values were not significantly affected by the diet and were similar to those observed by Brugiapaglia et al. for white veal meat of Holstein male calves [36].

Cooking loss and shear force were affected by the experimental diet. Hemp cake supplementation significantly increased cooking loss of the Longissimus dorsi muscle. The values found in this study were near the 28.6 [27] and 30.9% [14] reported in the literature for Holstein calves. The tenderness of the meat is the most important palatability characteristic for consumers [12,36]. Hemp cake supplementation negatively affected this parameter by increasing the shear force of the meat. Even though the values found in this study were higher than the 23.94N reported for white veal calves [27], this negative effect does not agree with the fact that intramuscular fat positively influences meat tenderness and, as both groups had similar intramuscular fat values, they should have had similar values.

Water, intramuscular fat, protein and iron content in the Longissimus dorsi muscle were not affected by the hemp cake supplementation. Moisture and protein values were similar to the ones found in the literature [14,37]. Intramuscular fat plays an important role in palatability and levels between 3.0 and 7.3 g/100 g of muscle have been generally considered acceptable for consumers in terms of visual quality and health concerns [14]. Considering this, the intramuscular fat (3.71 g/100 g muscle) values in this study were satisfactory but were higher than the 1.94% reported in Holstein bulls fed with concentrate [38] and the 0.65% reported in the same muscle of Holstein calves [34]. Owing to the young age of the animals, the diet and the fact that we are dealing with white veal meat, the iron concentration in the meat was very low (2.83 for the CTR and 2.95 mg/kg muscle for the HC group) and no significant difference between the two groups was observed. Values are near the ones reported in the literature for Holstein male calves slaughtered at 6 months with 3.77 mg of iron/kg of meat [36].

For both groups, and supported by the literature [14,39], oleic acid (C18:1) was the most abundant FA, followed by palmitic (C16:0) and stearic acid (C18:0). Our data showed that the HC group had higher proportions of oleic acid than the CTR group even though the HC diet had a lower quantity of this FA. On the one hand, similar values were reported for the oleic acid content, such as 38.64 in Holstein rib samples at a slaughter weight of 401–500 kg [15] and 39.64 in beef from steers fed with 9% of full-fat hemp seed [17], while on the other hand, literature findings state that feeding hemp oil and seed reduces levels of oleic acid in chicken breast meat [18] and also in beef [17]. The other two major FAs, palmitic and stearic, did not show any difference between treatments.

For PUFA, linoleic acid (C18:2) tended to predominate, but it was in the linolenic acid (C18:3) that a difference was found. It is well known that n-3 FAs such as linolenic acid have been recognized as being beneficial for human health. Values of 0.34 [39] and 0.53 g/100 g of total FA [15] were reported on male Holstein rib samples. In fact, including flaxseed [12] and hemp seed in diets has been shown to increase linolenic acid content within beef cuts [17]. However, in this trial the significance of a higher level in the CTR than the HC group was unexpected. This could be due to the low content of ALA (12.82 g/100 g of total FA) in the hemp cake used, as the average range reported is between 14.62 and 19.10 g/100 g of total FA [6]. For instance, the only experiment using hemp to improve the fatty profile of meat used full-fat hemp seed with an ALA content of 24.6 and found an improvement of +51.45% of this FA in the meat [17]. Beef is a natural source of CLA, and it is derived from dietary PUFA [16]. Supplementation of the diet with PUFA-rich oils or seeds has led to increases in CLA, but as normal concentrations are low the increases are not appreciable [16]. Typical concentrations are less than 1% of total FA [16], as was found in this study.

As regards the FA classes, there were no significant differences between the treatments. The FA distribution of white veal is similar to other meat products and was composed primarily by MUFAs (47.2%) and SFAs (45.3%) with very low amounts of PUFA (7.5%). The sum of n-3 FA, which was basically the quantity of ALA, was lower in the HC group (0.36) and it is also lower than the 0.96 reported for intramuscular fat in the longissimus muscle of German Holstein bulls fed with concentrate [39]. The lower n-6:n-3 ratio of the meat in the CTR group (16.77) in the current study indicates a healthier nutritional profile than the HC group (19.85). Both results were higher than the recommendation of 4 by the UK Department of Health and also seemed high in comparison to that of 2.1 given as an average for cattle meat [40].

4.6 Conclusions

Using hemp cake as an ingredient in the concentrate of Holstein veal calves can be considered safe since it did not affect the calves' health status or the in vivo performance parameters. However, average daily gain in the first period of the experiment and dressing percentage had an effect on the muscular growth with the hemp cake inclusion. Moreover, this inclusion did not change the meat color, which remained pale (low values of redness and yellowness) and for this reason still well accepted by consumers. Other approaches should be considered in order not to alter meat quality parameters such as cooking loss, shear force and the n-6:n-3 ratio. The enrichment of n-3 fatty acids of meat, obtained by the calves receiving hemp cake, was not successful due to a possible low percentage of hemp inclusion in the diet. Further experiments using higher doses are suggested to improve the fatty acid profile of meat.

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Chapter 5.

Evaluation of behavior in veal calves fed milk containing different levels of hempseed cake (*Cannabis sativa L*.)

5.1 Abstract

The present study aimed to evaluate the effect on behavioral patterns of veal calves fed with increasing levels of hempseed cake (HSC) diluted in the milk replacer. In total, 48 Belgian Blue veal calves (12 females and 36 males), with a body weight (BW) of 62.0 kg and age of 42.6 days, were offered the same type and quantity of solid and liquid feed two times a day but randomly assigned to one of the three different HSC inclusion levels: 0% (CTR), 3% (T3), and 6% (T6). The study lasted for 6 months until slaughter. During this time, their behavior was recorded using video cameras provided with a surveillance system. The results indicated that HSC had negligible effect on calves' behavior and that calves, in general, spend most of their time resting and ruminating as they normally do with conventional diets. Hempseed cake inclusion (T3 and T6) increased ($P < 0.05$) the appetite for solid food and licking behavior during the late afternoon. T3 female calves increased ($P < 0.05$) their movement in the late afternoon. Male calves decreased ($P < 0.05$) their positive interaction, movement, and cross-sucking in the late afternoon as the inclusion of HSC increased. The inclusion of HSC into veal calves' diet did not negatively affect the animal's behavior; therefore, it can be suggested as a novel ingredient.

5.2 Introduction

Cannabis sativa L., commonly known as hemp, has been primarily grown for its fiber (1), but over the last 10 years, it has also been attracting some interest in the animal feeding sector. Hemp by-products, such as oil, seeds, and cake, have large amounts of polyunsaturated essential fatty acids, particularly linoleic and alpha-linolenic, which make them suitable ingredients for the formulation of animal feeds. Fish and farm animals such as laying hens, broiler chickens, pigs, sheep, and other ruminants are continuously being tested in order to establish the range of inclusion levels that ensures animal health and also leads to optimal performances (2).

An interesting farm production system is that of white veal meat. Italy is the fifth major producer of this type of meat in the European Union, owning a population of 378,459 calves in 2022 (3). White veal calves must be younger than 8 months old at slaughter and are raised to obtain pale meat based on a low-iron milk replacer with the addition of concentrate (4). For this purpose, the use of hempseed cake (HSC) in their diets could represent a good protein source.

Although industrial hemp is characterized by having trace amounts (<0.30%) of tetrahydrocannabinol (THC) (5), which has psychotropic effects, it cannot cause intoxication. Cannabidiol (CBD) is the second bioactive compound in hemp, and it is known for having remarkable applications without psychoactive effects (6). In fact, CBD extract from C. sativa has been tested in dogs and seems to reduce aggressive behavior (7). Moreover, chronic treatment with non-psychotropic C. sativa caused no alterations in body weight, movement, or anxiety in mice, while increasing pro-social behavior (8). Considering that research on the behavioral effects of hemp is fairly recent, no studies have focused on farm animals to date. In addition, since consumers, at present, are increasingly concerned about animal welfare issues (9), it is always necessary to test new feed sources in order to understand any possible behavioral changes before the feed becomes part of the calves' diet. This study aimed to evaluate the effect on behavior patterns of veal calves fed with increasing levels of hempseed cake diluted in the milk replacer.

5.3 Materials and Methods

All procedures were performed according to the Italian legislation on animal care and approved by the Ethical Committee for the care and use of experimental animals at the University of Padova, which operates within the European Directive 86/609/CEE regarding the protection of animals used for experimental and other scientific purposes (approval number 74/2022).

5.3.1 Housing system and experimental design

This trial was carried out at a commercial farm (BE farm, owned by Barban Elia, Castelfranco, TV, Italy) and aimed at evaluating the differences in behavioral patterns when Belgian blue calves were fed with increasing levels of hempseed cake in the milk replacer. Data for this study were obtained from 6 months of the fattening period of 48 Belgian Blue veal calves (12 females and 36 males). The number of male and female calves was chosen after sample size calculation using a Student's T-test with a statistical power of 0.75 and a statistical significance of a = 0.05. The animals were randomly distributed in 12 pens (four calves per pen); after the random assignment, some changes were made to balance the pens for body weight (BW). There were nine pens (three per treatment) for male and three for female (one per treatment) calves. Each pen had a dimension of 3 × 2.5 m. The average age on arrival was 42.6 ± 9.5 days, and the average BW was 62.0 ± 4.5 kg. Animals were reared for 6 months until slaughter.

The diet consisted of solid and liquid feed (Table 1), and all animals received the same amount of each feed. The solid feed used was Fibra Flakes 314 PN concentrate (Veneta Fiocchi, Riese Pio X, TV, Italy). The liquid feed followed the typical program used for white veal calves by the farm, where the trial was conducted and consisted of six types of milk replacers (Sofivo, Maen Roch, France) that were offered throughout the experiment (Figure 1) until weaning, from 340 g on day 1 to 1,213 g before slaughter in the sixth month of the trial. The three experimental diets (CTR, T3, and T6) differed in the percentage of hempseed cake (as feed) diluted in the milk replacer and were based on the EFSA daily recommendation for hemp by-products in ruminants (2). The CTR group had no inclusion of HSC. T3 had an inclusion of 3% (as feed) of HSC, corresponding to 7.5 g on day 1 and 750 g before slaughter. T6 had 6% (as feed) of HSC inclusion, corresponding to 16 g on day 1 and 1,500 g before slaughter. The chemical composition (% of DM) of the HSC was 26.17 of CP, 11.89 of EE, 11.89 of CF, 0.014 of THC, and 27.31 of iron (mg/kg DM). Liquid feed was prepared freshly before each meal by mixing the milk replacer with water and then with the HSC. It was provided two times a day (at 6.00 a.m. and 5.00 p.m.) on individual teat buckets placed in each pen. Solid feed was always provided after the liquid feed and also twice a day in increasing amounts from 75 g on day 1 to 2,400 g at the end of the trial. Fresh water was offered ad libitum using a drinking cup placed in the corner of each pen.

Table 1. Chemical composition of the concentrate (CON) and the six milk replacers (MR) given to all the calves

Item	CON	MR1	MR2	MR3	MR4	MR5	MR6
Chemical composition, % of DM							
Dry Matter	88.80	96.5	95.84	95.69	96.16	96.01	96.54
Crude Protein	14.49	19.88	21.40	21.72	17.20	23.46	21.18
Ether Extract	4.07	16.50	22.99	23.27	16.74	20.31	21.93
Non-structural Carbohydrates	77.98	56.67	48.31	47.81	58.48	49.14	50.45
Ash	3.46	6.95	7.30	7.21	7.59	7.09	6.44
Iron (mg/kg)	55.83	50.4	7.21	6.39	28.36	26.04	6.12

CON = Concentrate; MR = Milk replacer; MR1 = Elvor Demarrage 50; MR2 = Zoogamma M-21; MR3 = Elvor Ingrasso 50; MR4 = Unico Super I; MR5 = Top 60; MR6 = Elvor Finition 50 N.

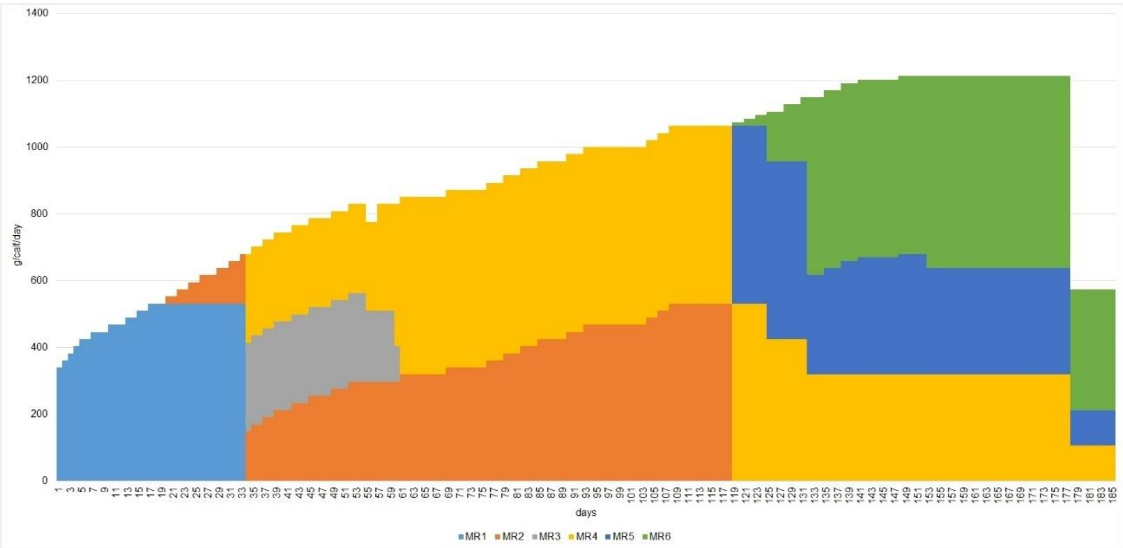

Figure 1. Milk replacers (MR1, Elvor Demarrage 50; MR2, Zoogamma M-21; MR3, Elvor Ingrasso 50; MR4, Unico Super I; MR5, Top 60; MR6, Elvor Finition 50N) intake (g/calf/day) during the experiment.

5.3.2 Behavioral observations

The behavior of the calves was recorded using a video surveillance system [H.264 Standalone Digital Video Recorder (DVR); Atlantis, Atlantis-land, MI, Italy]. A total of 12 cameras were fixed on the ceiling in front of each pen in order to cover the whole area. The cameras recorded 24 h of 3 days per week from the beginning of the trial, but only 1 day per week (the same day each week) was chosen for this study. The videos were analyzed by two different viewers using the Playback Software program (Atlantis, Atlantis-land, MI, Italy), which allowed them to observe the behavior of the animals during the entire target day. In total, 432 h were analyzed (216 h per viewer). They were taken for 18 days, starting approximately a month after the administration of the experimental diets. The ethogram was built by looking at three sample hours of the whole day and considering the behaviors found in the veal calves during the trial. Behaviors were classified according to the literature (10), and a total of 24 behaviors were identified (Table 2). Each behavior was ascribed to one of the following categories: ingestion (three behaviors), resting (three), ruminating (three), movement (four), environmental interaction (three), positive interaction (four), negative interaction (three), and self-grooming (one). A letter (A, B, C, or D) was assigned to each calf within a pen for unique identification. After a previous validation of the scan sampling interval, every 5 min, the behavior of each calf was registered in an Excel spreadsheet. To appreciate the variability of the behaviors over the 24 h of a day, the day was divided into six time intervals (timings) of 4 h each: night (NI, 12.00 midnight−4.00 a.m.), early morning (EM, 4.00 a.m.−8.00 a.m.), late morning (LM, 8.00 a.m.−12 noon), early afternoon (EA, 12.00 noon−4.00 p.m.), late afternoon (LA, 4.00 p.m.−8.00 p.m.), and evening (EV, 8.00 p.m.−12.00 midnight). Each behavior was considered as a trait and was expressed as the percentage of recordings of such behavior collected from an individual within a target timing. Specifically, the number of occurrences of a specific behavior recorded for each calf within a specific timing of a target day was divided by the total number of recording occurrences within a timing and then multiplied by 100. In such a way, each individual behavior was expressed as a percentage of recordings within the respective timing. The final dataset included 5,184 records of each individual single behavior within a day of observation and specific timing.

Table 2. Ethogram of calves' behavior within categories and main description of each behavior

Behavioral categories	Behavior	Description
Ingestion	Eating solid	Ingestion of the solid feed from the feeder
	Eating liquid	Ingestion of the milk replacer
	Drinking	Drinks from the drinking cup in the corner of the pen
Resting	Sternal recumbency	Resting or sleeping with the legs curled under the body and the head up
	Lateral recumbency	Resting or sleeping with the legs and head outstretched
	Rest standing	Standing inactive in a relaxed posture; head lowered, eyes partially or totally closed
Ruminating	Standing ruminating	Chewing motions of teeth while standing on all 4 legs
	Lateral ruminating	Ruminating with the legs and head outstretched
	Sternal ruminating	Ruminating with the legs curled under the body and the head up
Movement	General agitation	Walking beside the feeder from one side to another with or without a reason, mostly close to the feeding times
	Standing/Lying	Passing from standing to lying or viceversa
	Moving	Displacement slowly from one location to another inside the pen
	Running	Rapid movement with constant changes of direction inside the pen
Environmental interaction	Olfactory investigation	Sniffing various parts of another individual's head or body; typically begins after a nose-to-nose approach
	Object playing	Playing with an object inside the pen
	Licking	Licking the wall, the empty feeder or some object inside the pen
Positive interaction	Mutual grooming	Grooming and licking another individual using gentle gestures
	Playing	Playing with another calf while making physical contact with their body parts, eventually pushing each other without force
	Pen interaction	Staying near the adjacent pen and exploring by licking the calves there
	Sexual behavior	Mounting. Jumps to lift both forelegs onto the rump of another calf
Negative interaction	Cross-sucking	Sucking or licking the perianal zone of another calf
	Stereotypies	Repetitive or unnatural movements with a relative regularity without any apparent function (ex. tongue playing/rolling, bar biting)
	Head butting	Two calves butting each other with their foreheads and sticking together for some seconds
Self grooming		A calf licking any part of itself
Non visible		Not visible from the camera or hidden behind other calves

5.3.3 Statistical analysis

The trial focused on the expression of a specific behavior for each diet (CTR, T3, and T6). Each calf represented an experimental unit, and all behaviors were expressed as fractions of an hour. An effect size correlation of r = 0.081 was calculated by comparing control vs. treatment on some behaviors relevant to calf wellbeing, such as cross-sucking, using Cohen's d statistics. A separate analysis for each behavior of an individual within each target timing of 4 h (6 timings × 4 h = 24 h) was performed as dependent variables of a mixed model [mixed procedure; SAS, SAS/STAT User's Guide (SAS Institute: Cary, NC, USA, 2013)] in a linear model analysis as written below:

$$Y_{ijklm} = \mu + day_i + D_j + T_k + S_l + (DxT)_{jk} + (DxS)_{jl} + (TxS)_{kl} + (DxTxS)_{jkl} + ID_m + e_{ijklmn},$$

Where Y_{ijklm} is the target individual behavior as a dependent variable and μ is the overall mean. The fixed effects were the day of observation (18 levels, corresponding to each target day), the diet (D: CTR, T3, and T6), the timing (T: EM, LM, EA, LA, E, and N), and sex (S: M or F). The interactions among D, T, and S were also considered. Random effects included the identity of each individual calf (ID) and the error (e). The effect of the two viewers was not included as a separate effect in the final model because it was already considered within the effect of the day, as each day was entirely observed by a single viewer. All the variables and their residuals were tested for normality using a Shapiro–Wilk test. As post-hoc analysis of the mixed model, the least square means [LS means option; MIXED procedure, SAS, SAS/STAT User's Guide (SAS Institute: Cary, NC, USA, 2013)] were calculated for each effect included. Additionally, the comparisons between each pair of levels for the LS means of each fixed effect were made using the Student's t-test analysis. A Bonferroni correction was done to make it as conservative as possible SAS, SAS/STAT User's Guide (SAS Institute: Cary, NC, USA, 2013). A P-value of <0.05 was used to indicate statistical significance.

5.4 Results

None of the calves developed diseases severe enough to be a reason for exclusion from the study. The descriptive values of the duration of the calves' behaviors are reported in Table 3. During the experiment, some behaviors were identified as belonging to some specific timings. In general, resting and ruminating were the two main behaviors performed by the calves throughout the day. The ingestion behavior was observed mostly during the day. The calves spent 8.4% of their time eating solid food in the late morning and 9.9% in the late afternoon after the administration of the two meals (6.00 a.m. and 5.00 p.m.). As regards the resting behavior, both sternal and lateral recumbency appeared predominantly during the night when the calves just lay down inside the pen. Sternal recumbency was observed in the late morning (38.6%) and early afternoon (40.5%), especially in the evening (53.1%) and during the night (45%). On the other hand, standing resting was barely observed in the evening (5.9%) and night (2.1%), as the calves preferred to rest in the recumbent position. The most common ruminating behavior was sternal ruminating, occurring mainly during the night (22.9%), where the animals remained in sternal recumbency. The environmental interaction of the calves was mostly represented by olfactory investigation and licking. Olfactory investigation toward the other pen mates was observed mainly during the late morning (6.4%) and late afternoon (9.7%). Generally, this behavior occurred at the end of the meal as did licking. The negative interaction behavior in which calves spent the most time was cross-sucking, usually performed in the early morning (1.3%), before the first meal, and in the late afternoon (1.8%) after the evening meal. The other negative behaviors were not strictly linked to a specific time slot but were displayed throughout the day. A few stereotypies were also observed in all the time slots considered.

The mixed-model analysis (Table 4) shows the incidence of the main effects (diet, sex, and timing) and their interactions for each target behavior (Table 5). Diet-influenced ($P < 0.05$) behaviors include eating liquid, running, cross-sucking, licking, olfactory investigation, and mutual grooming. Sex influenced ($P < 0.05$) eating liquid, sternal recumbency, lateral recumbency, sternal ruminating, moving, running, licking, and sexual behavior. Timing influenced ($P < 0.05$) all behaviors.

Figures 2–6 report the most relevant interactions among the main effects that were significant ($P < 0.05$). The inclusion of 3% and 6% of HSC significantly increased the time spent running and licking (Figure 2) and decreased the amount of time carrying out olfactory investigation and cross-sucking (Figure 3). Both sexes expressed more time cross-sucking during the early morning and late afternoon, but mostly in the latter after the second milk administration. Male calves reduced the time spent on cross-sucking as the HSC inclusion increased in the diet. For female calves, this reduction only happened for the T3 group. Meanwhile, the T6 and CTR groups were not influenced by the diet (Figure 3).

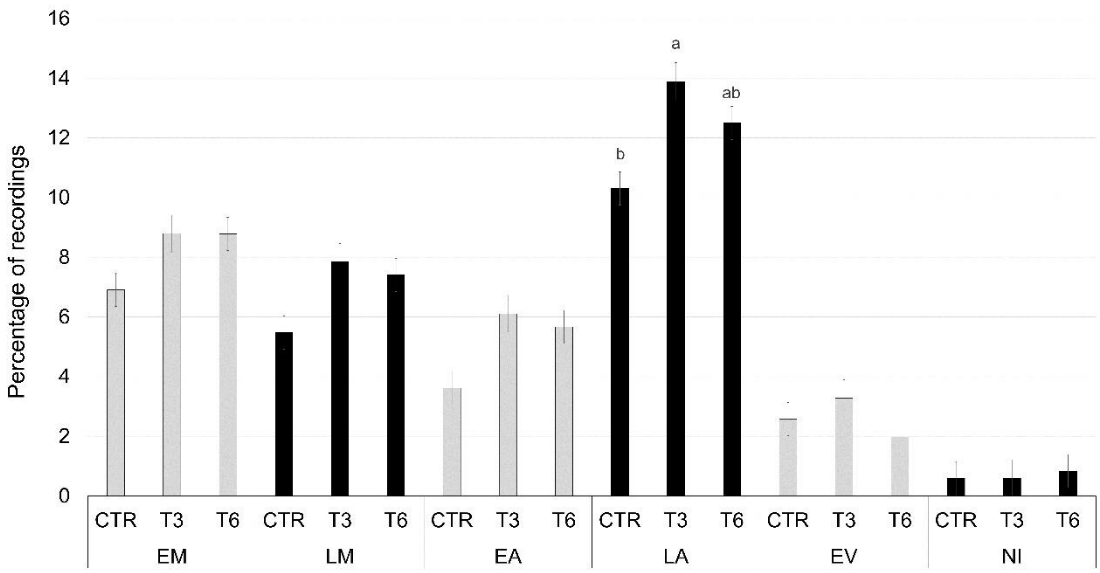

Figure 2. Least square means of the interaction of diet (CTR, 0%of HSC; T3, 3%of HSC; T6, 6% of HSC), and timing (EM, early morning; LM, late morning; EA, early afternoon; LA, late afternoon; EV, evening; NI, night) on licking behavior expressed as a percentage of recordings. Black lines represent SE. Different letter differ statistically ($P < 0.05$)

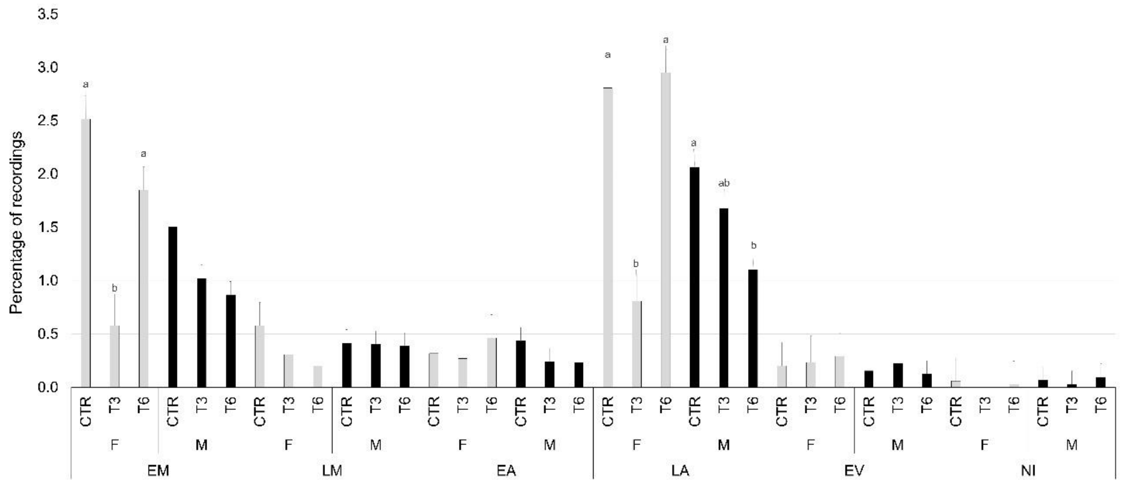

Figure 3. Least square means of the interaction of diet (CTR, 0%of HSC; T3, 3%of HSC; T6, 6% of HSC), and timing (EM, early morning; LM, late morning; EA, early afternoon; LA, late afternoon; EV, evening; NI, night) on cross-sucking behavior expressed as a percentage of recordings. Black lines represent SE. Different letter differ statistically (P < 0.05)

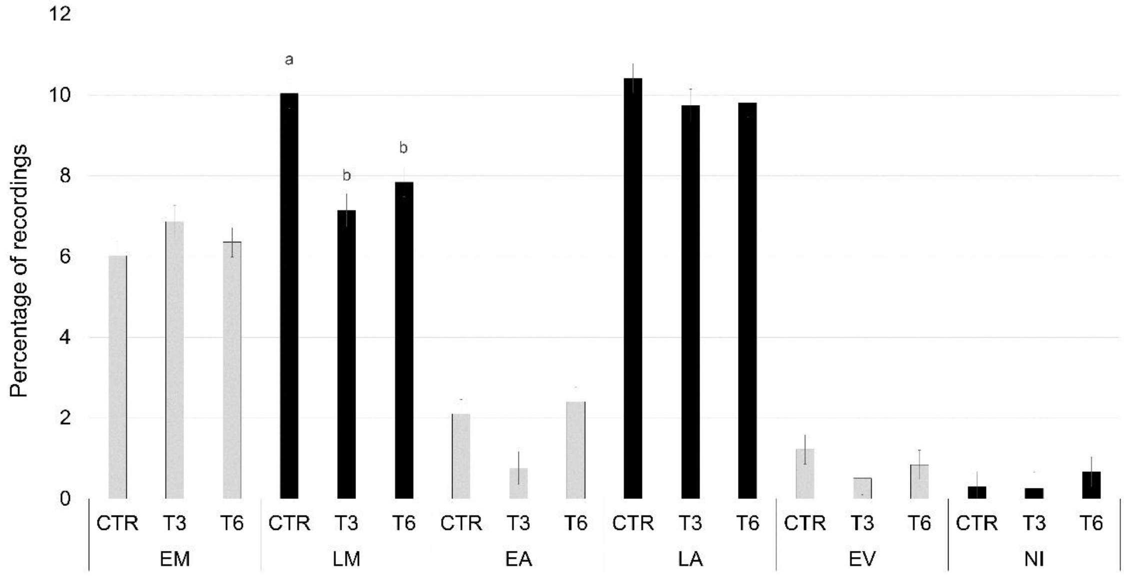

Figure 4. Least square means of the interaction of diet (CTR, 0%of HSC; T3, 3%of HSC; T6, 6% of HSC), and timing (EM, early morning; LM, late morning; EA, early afternoon; LA, late afternoon; EV, evening; NI, night) on eating solid behavior expressed as a percentage of recordings. Black lines represent SE. Different letter differ statistically (P < 0.05)

Table 3. Least square means of percentage of recordings of calves' behavior along the day, calculated as the ratio among the number of occurrences of a target behavior and the total recording occurrences in a day and then multiplied by 100.

Behavior	Timing [1]					
	EM	LM	EA	LA	EV	NI
Ingestion						
Eating solid	6.13 ± 5.43	8.36 ± 7.38	1.64 ± 3.42	9.93 ± 5.14	0.93 ± 2.03	0.47 ± 1.48
Eating liquid	2.04 ± 1.35	0.00 ± 0.00	0.00 ± 0.00	2.02 ± 1.35	0.00 ± 0.00	0.00 ± 0.00
Drinking	0.15 ± 0.65	0.29 ± 1.00	0.16 ± 0.68	0.27 ± 0.91	0.14 ± 0.69	0.06 ± 0.37
Resting						
Sternal recumbency	28.87 ± 14.42	38.58 ± 17.14	40.45 ± 18.79	17.47 ± 11.37	53.14 ± 21.63	44.85± 21.68
Lateral recumbency	6.60 ± 9.97	7.29 ± 11.20	12.56 ± 14.37	2.31 ± 5.17	12.19 ± 16.41	14.97 ± 17.32
Rest standing	14.48 ± 9.61	11.07 ± 9.11	5.38 ± 5.33	21.37 ± 12.25	5.85 ± 6.70	2.11 ± 2.79
Ruminating						
Standing ruminating	0.16 ± 0.75	0.10 ± 0.68	0.05 ± 0.35	0.13 ± 0.73	0.03 ± 0.32	0.05 ± 0.35
Lateral ruminating	2.59 ± 4.73	1.14 ± 3.54	3.14 ± 5.33	0.56 ± 2.16	1.64 ± 3.88	5.68 ± 8.85
Sternal ruminating	13.00 ± 9.74	7.31 ± 7.41	17.24 ± 11.95	6.09 ± 6.08	9.52 ± 9.18	22.94 ± 15.52
Movement						
General agitation	0.18 ± 0.59	0.04 ± 0.33	0.01 ± 0.14	0.40 ± 0.87	0.03 ± 0.26	0.00 ± 0.00
Standing/Lying	0.20 ± 0.67	0.22 ± 0.67	0.20 ± 0.63	0.17 ± 0.61	0.29 ± 0.76	0.19 ± 0.65
Moving	0.69 ± 1.39	0.80 ± 1.41	0.42 ± 1.05	1.42 ± 2.05	0.40 ± 0.96	0.12 ± 0.53
Running	0.02 ± 0.21	0.08 ± 0.45	0.03 ± 0.28	0.28 ± 0.81	0.05 ± 0.35	0.00 ± 0.00
Environmental interaction						
Olfactory investigation	5.86 ± 5.22	6.40 ± 5.64	4.44 ± 4.74	9.68 ± 8.05	3.51 ± 4.58	1.50 ± 2.17
Object playing	0.17 ± 0.90	0.08 ± 0.5	0.05 ± 0.35	0.16 ± 0.72	0.01 ± 0.17	0.00 ± 0.00
Licking	7.61 ± 5.72	6.42 ± 5.59	4.55 ± 5.09	11.69 ± 7.52	2.54 ± 3.66	0.72 ± 1.69
Positive interaction						
Mutual grooming	1.44 ± 2.18	1.75 ± 2.44	1.23 ± 2.03	2.30 ± 2.83	0.79 ± 1.66	0.55 ± 1.70
Playing	0.23 ± 0.77	0.41 ± 1.20	0.16 ± 0.67	0.90 ± 1.84	0.15 ± 0.59	0.02 ± 0.21
Pen interaction	0.89 ± 1.79	0.78 ± 1.62	0.42 ± 1.22	1.42 ± 2.18	0.18 ± 0.69	0.02 ± 0.23
Sexual behavior	0.02 ± 0.19	0.02 ± 0.21	0.01 ± 0.20	0.08 ± 0.43	0.01 ± 0.18	0.00 ± 0.07
Negative interaction						
Cross-sucking	1.28 ± 2.13	0.39 ± 1.01	0.32 ± 0.96	1.78 ± 2.62	0.18 ± 0.64	0.06 ± 0.38
Stereotypies	0.21 ± 0.90	0.24 ± 0.97	0.31 ± 0.97	0.41 ± 1.53	0.17 ± 0.67	0.15 ± 0.76
Head butting	0.54 ± 1.17	0.93 ± 1.62	0.28 ± 0.84	1.60 ± 2.15	0.34 ± 0.97	0.04 ± 0.28
Self grooming	2.32 ± 2.57	2.96 ± 2.59	3.26 ± 3.35	3.59 ± 3.30	2.43 ± 3.02	1.94 ± 2.50
Non visible	4.32 ± 18.63	4.22 ± 19.34	3.81 ± 18.29	3.97 ± 18.20	5.45 ± 20.98	3.55 ± 17.99

EM = Early morning; LM = Late morning; EA = Early afternoon; LA = Late afternoon; EV = Evening; NI = Night. [1] Time slots

Table 4. ANOVA reporting the F-statistics and P-values of all the main effects and interactions of all behaviors expressed as percentage of recordings

	Day		Diet (D)		Sex (S)		Timing (T)		D * S		D * T		S * T		D * S * T	
Behavior	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Ingestion																
Eating solid	11.85	<0.001	2.54	0.091	0.87	0.352	518.56	<0.001	4.32	0.013	5.14	<0.001	1.73	0.124	1.87	0.044
Eating liquid	15.35	<0.001	12.15	0.000	34.70	<0.001	1441.77	<0.001	8.81	<0.001	11.52	<0.001	33.18	<0.001	6.37	<0.001
Drinking	34.45	<0.001	1.82	0.174	0.18	0.675	12.51	<0.001	1.76	0.171	1.38	0.184	1.95	0.083	1.27	0.240
Resting																
Sternal recumbency	52.82	<0.001	0.37	0.690	4.76	0.029	400.54	<0.001	1.91	0.148	1.14	0.329	4.55	<0.001	0.89	0.543
Lateral recumbency	86.53	<0.001	2.38	0.105	9.67	0.002	94.87	<0.001	0.31	0.734	2.68	0.003	2.37	0.037	0.81	0.616
Rest standing	60.85	<0.001	1.32	0.279	0.16	0.692	614.74	<0.001	0.10	0.904	4.19	<0.001	0.94	0.456	1.57	0.108
Ruminating																
Standing ruminating	8.35	<0.001	1.02	0.371	0.16	0.691	4.20	0.001	1.70	0.182	1.65	0.087	2.21	0.051	1.47	0.144
Lateral ruminating	31.69	<0.001	1.04	0.362	1.45	0.229	87.23	<0.001	1.02	0.360	1.88	0.044	0.70	0.620	2.63	0.003
Sternal ruminating	45.27	<0.01	0.41	0.668	4.52	0.034	350.33	<0.001	0.70	0.498	4.88	<0.001	9.57	<0.001	1.58	0.105
Movement																
General agitation	6.51	<0.001	2.39	0.105	2.06	0.152	61.05	<0.001	0.47	0.626	1.59	0.103	2.41	0.034	5.13	<0.001
Standing/Lying	2.68	<0.001	0.15	0.862	2.76	0.097	3.50	0.004	0.27	0.761	1.14	0.325	1.12	0.345	1.78	0.059
Moving	27.49	<0.001	0.12	0.887	4.50	0.034	98.69	<0.001	3.65	0.026	1.81	0.054	4.77	<0.001	2.48	0.006
Running	3.79	<0.001	5.05	0.011	17.61	<0.001	59.83	<0.001	4.13	0.016	3.51	<0.001	10.39	<0.001	4.24	<0.001
Environmental interaction																
Olfactory investigation	91.16	<0.001	3.82	0.030	0.47	0.492	228.15	<0.001	1.38	0.251	2.37	0.009	0.76	0.579	1.77	0.060
Object playing	1.66	0.043	1.59	0.217	0.04	0.836	9.07	<0.001	5.05	0.006	1.03	0.416	0.97	0.432	1.46	0.148
Licking	20.57	<0.001	3.71	0.033	4.95	0.026	451.73	<0.001	1.81	0.164	5.24	<0.001	6.61	<0.001	3.23	<0.001
Positive interaction																
Mutual grooming	10.82	<0.001	3.42	0.042	0.48	0.487	54.51	<0.001	0.77	0.464	0.25	0.991	1.30	0.261	1.32	0.214
Playing	31.09	<0.001	0.80	0.456	1.32	0.251	57.59	<0.001	0.66	0.518	1.68	0.080	0.51	0.769	0.57	0.843
Pen interaction	32.87	<0.001	0.75	0.477	1.96	0.161	99.07	<0.001	10.30	<0.001	1.56	0.111	1.62	0.151	4.45	<0.001

continue																
Sexual behavior	2.38	0.001	1.14	0.329	5.69	0.017	3.55	0.003	2.80	0.061	1.03	0.412	4.59	<0.001	0.90	0.531
Negative interaction																
Cross-sucking	18.21	<0.001	5.51	0.008	3.15	0.076	166.64	<0.001	3.91	0.020	8.54	<0.001	5.77	<0.001	8.31	<0.00
Stereotypies	5.62	<0.001	0.77	0.471	0.00	0.961	5.83	<0.001	0.06	0.939	1.11	0.349	0.34	0.888	0.82	0.605
Head butting	23.06	<0.001	0.34	0.712	1.38	0.240	118.59	<0.001	0.59	0.554	1.22	0.271	1.07	0.375	0.88	0.556
Self grooming	15.86	<0.001	0.34	0.711	13.80	<0.001	34.63	<0.001	1.66	0.191	1.18	0.298	1.50	0.187	1.56	0.113

Table 5. Least square means of behaviors, expressed as percetange of recordings, for the main effects considered in the ANOVA

	Diet				Sex			Timing[1]						
Behavior	CTR	T3	T6	SE	F	M	SE	EM	LM	EA	LA	EV	NI	SE
Ingestion														
Eating solid	5.016	4.211	4.655	0.247	4.760	4.494	0.194	6.412[c]	8.343[b]	1.754[d]	9.989[a]	0.855[e]	0.410[e]	0.219
Eating liquid	0.701[b]	0.844[a]	0.648[b]	0.028	0.827[a]	0.635[b]	0.022	2.125[b]	0.000[c]	0.000[c]	2.260[a]	0.000[c]	0.000[c]	0.032
Drinking	0.253	0.125	0.172	0.048	0.195	0.172	0.037	0.135[b]	0.289[b]	0.159[a]	0.318[a]	0.140[b]	0.059[b]	0.038
Resting														
Sternal recumbency	38.81	38.22	37.24	1.344	39.79[a]	36.40[b]	1.055	29.50[d]	40.49[c]	40.74[c]	17.34[e]	54.64[a]	45.86[b]	0.979
Lateral recumbency	10.00	7.945	7.130	0.994	6.573[b]	10.15[a]	0.780	5.893[c]	6.242[c]	11.58[b]	1.92[d]	10.89[b]	13.63[a]	0.707
Rest standing	10.81	10.190	8.797	0.931	9.719	10.14	0.730	14.33[b]	10.69[c]	5.371[d]	21.36[a]	5.752[d]	2.09[e]	0.598
Ruminating														
Standing ruminating	0.070	0.113	0.076	0.022	0.081	0.091	0.017	0.137[a]	0.074[a]	0.059[a]	0.149[a]	0.032[b]	0.064[a]	0.024
Lateral ruminating	2.687	2.388	1.848	0.432	2.007	2.608	0.339	2.508[b]	1.048[c]	2.911[b]	0.481[d]	1.397[c]	5.501[a]	0.306
Sternal ruminating	12.72	13.97	13.35	0.963	14.53[a]	12.16[b]	0.756	13.60[c]	7.583[e]	18.21[b]	6.129[e]	9.870[d]	24.69[a]	0.655
Movement														
General agitation	0.108	0.124	0.083	0.013	0.094	0.116	0.010	0.158[c]	0.064[b]	0.006[c]	0.379[a]	0.021[c]	0.000[b]	0.019
Standing/Lying	0.223	0.236	0.216	0.025	0.249	0.201	0.019	0.230[a]	0.228[a]	0.223[a]	0.158[b]	0.312[a]	0.199[b]	0.028
Moving	0.666	0.715	0.684	0.068	0.772[a]	0.604[b]	0.054	0.723[b]	0.826[b]	0.494[c]	1.580[a]	0.395[c]	0.111[d]	0.061
Running	0.071[b]	0.126[a]	0.091[a]	0.012	0.125[a]	0.066[b]	0.010	0.018[c]	0.093[b]	0.026[b]	0.357[a]	0.082[b]	0.000[c]	0.017
Environmental interaction														
Olfactory investigation	5.716[a]	5.271[a]	4.510[b]	0.324	5.037	5.294	0.254	5.686[b]	6.428[b]	4.408[c]	9.700[a]	3.376[d]	1.395[e]	0.254
Object playing	0.047	0.100	0.099	0.025	0.085	0.079	0.019	0.154[a]	0.085[a]	0.066[b]	0.159[a]	0.026[b]	0.000[b]	0.024
Licking	4.913[b]	6.753[a]	6.195[a]	0.489	6.583[a]	5.325[b]	0.384	8.161[b]	6.909[c]	5.131[d]	12.23[a]	2.611[e]	0.678[f]	0.334
Positive interaction														
Mutual grooming	1.644	1.234	1.030	0.175	1.232	1.373	0.138	1.315[b]	1.661[b]	1.210[c]	2.294[a]	0.797[d]	0.540[d]	0.127

continue														
Playing	0.284	0.338	0.289	0.032	0.282	0.325	0.025	0.230^{c}	0.407^{b}	0.161^{c}	0.862^{a}	0.141^{c}	0.021^{d}	0.041
Pen interaction	0.576	0.711	0.682	0.082	0.723	0.590	0.065	0.905^{b}	0.862^{b}	0.450^{c}	1.508^{a}	0.188^{d}	0.027^{d}	0.069
Sexual behavior	0.029	0.010	0.014	0.009	0.005^{b}	0.031^{a}	0.007	0.018^{a}	0.014^{b}	0.010^{b}	0.053^{a}	0.010^{b}	0.002^{b}	0.010
Negative interaction														
Cross-sucking	0.927^{a}	0.483^{ab}	0.716^{a}	0.093	0.804	0.614	0.073	1.390^{b}	0.383^{c}	0.326^{c}	1.902^{a}	0.204^{c}	0.047^{d}	0.076
Stereotypies	0.169	0.172	0.394	0.150	0.241	0.249	0.118	0.199^{b}	0.249^{a}	0.304^{a}	0.384^{a}	0.177^{b}	0.156^{b}	0.093
Head butting	0.592	0.641	0.568	0.062	0.558	0.642	0.049	0.539^{c}	0.852^{b}	0.256^{d}	1.595^{a}	0.322^{d}	0.040^{e}	0.059
Self grooming	2.854	3.068	3.077	0.220	3.472	2.527	0.173	2.490^{c}	3.320^{b}	3.562^{a}	3.837^{a}	2.664^{c}	2.125^{d}	0.164

CTR = Control; T3 = 3% of hempseed cake inclusion; T6 = 6% of hempseed cake inclusion; F = Female; M = Male; EM = Early morning; LM = Late morning; EA = Early afternoon; LA = Late afternoon; EV = Evening; NI = Night; SE = Standard error. [1] Time slots. $^{a\text{-}f}$ Values within a row with different superscripts differ significantly at $P < 0.05$.

Females spent significantly greater time than males eating liquid, resting, ruminating in sternal recumbency, moving, running, and licking, whereas males spent longer duration in lateral recumbency and sexual behavior. The CTR group spent more time eating solids in the late morning than T3 and T6 (Figure 4). Calves that received the diet with the HSC inclusion finished all the solid feed right after the first meal, whereas the CTR group always had some leftovers in the feeder, and they tended to eat more slowly. The diet did not influence the positive interaction behavior, but sex and timing did (Figure 5). This behavior was observed, in particular, during the late afternoon. For this time period, male calves decreased the time of positive interaction inside the pen when the HSC was included in the diet, whereas the same effect was not noticed in the female calves, which spent the same amount of time interacting positively regardless of the diet. During this trial, both male and female calves showed a different time budget for movement behavior (Figure 6). Even though both female and male calves were more active during the late afternoon, the HSC inclusion had opposite effects on the different sexes. Female calves of T3 and T6 spent more time (P < 0.05) on movement than those in the CTR group. On the contrary, male calves decreased (P < 0.05) their time spent on movement when the HSC inclusion increased in the diet. Although sexual behavior was the least noticed of the positive interaction behaviors, there was a statistical difference between sexes (Figure 7), showing that male calves expressed more sexual behavior than females, mostly in the late afternoon.

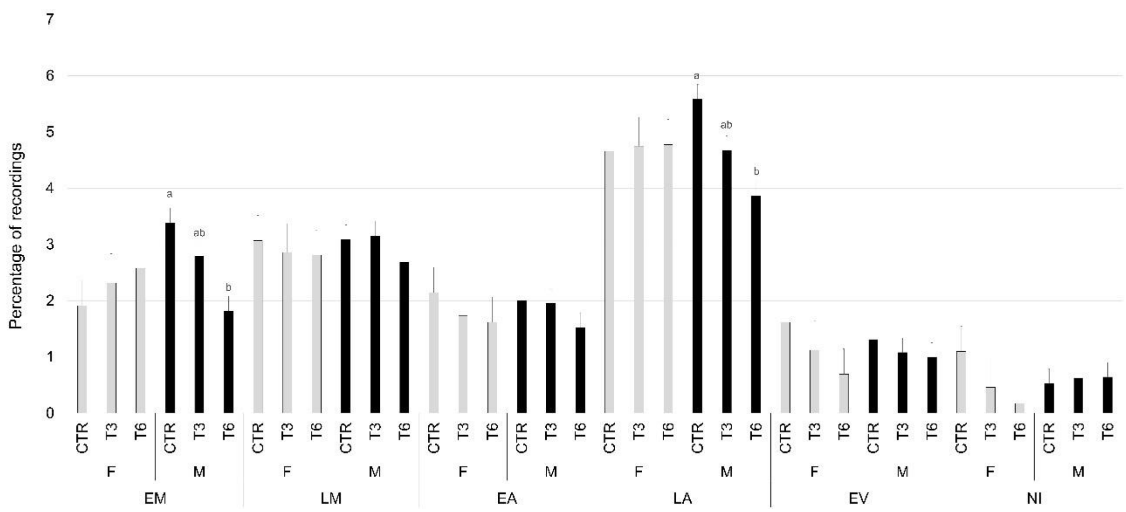

Figure 5. Least square means of the interaction of diet (CTR, 0%of HSC; T3, 3%of HSC; T6, 6% of HSC), and timing (EM, early morning; LM, late morning; EA, early afternoon; LA, late afternoon; EV, evening; NI, night) on positive interaction behavior expressed as a percentage of recordings. Black lines represent SE. Different letter differ statistically (P < 0.05)

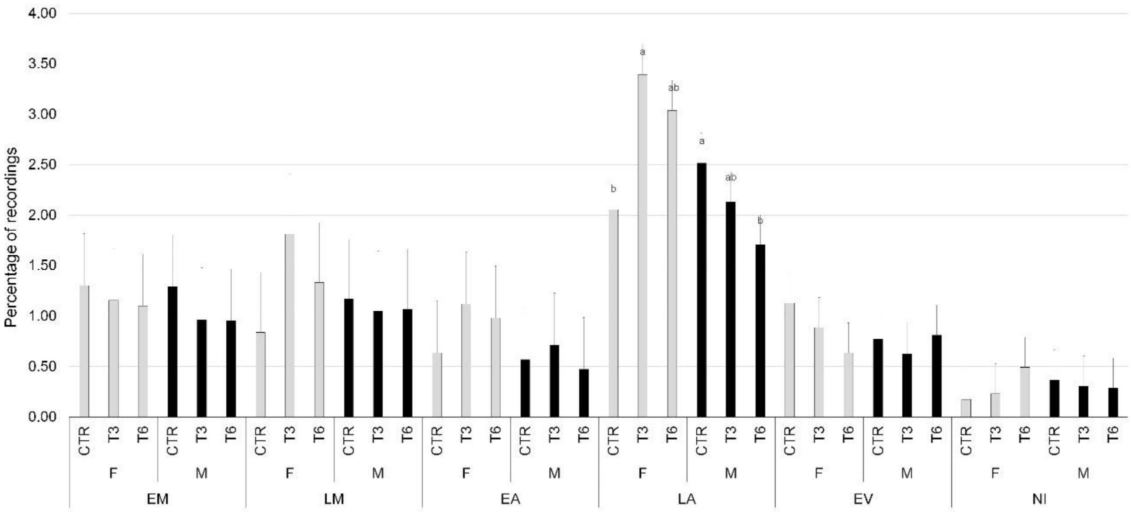

Figure 6. Least square means of the interaction of diet (CTR, 0%of HSC; T3, 3%of HSC; T6, 6% of HSC), and timing (EM, early morning; LM, late morning; EA, early afternoon; LA, late afternoon; EV, evening; NI, night) on movement behavior expressed as a percentage of recordings. Black lines represent SE. Different letter differ statistically (P < 0.05)

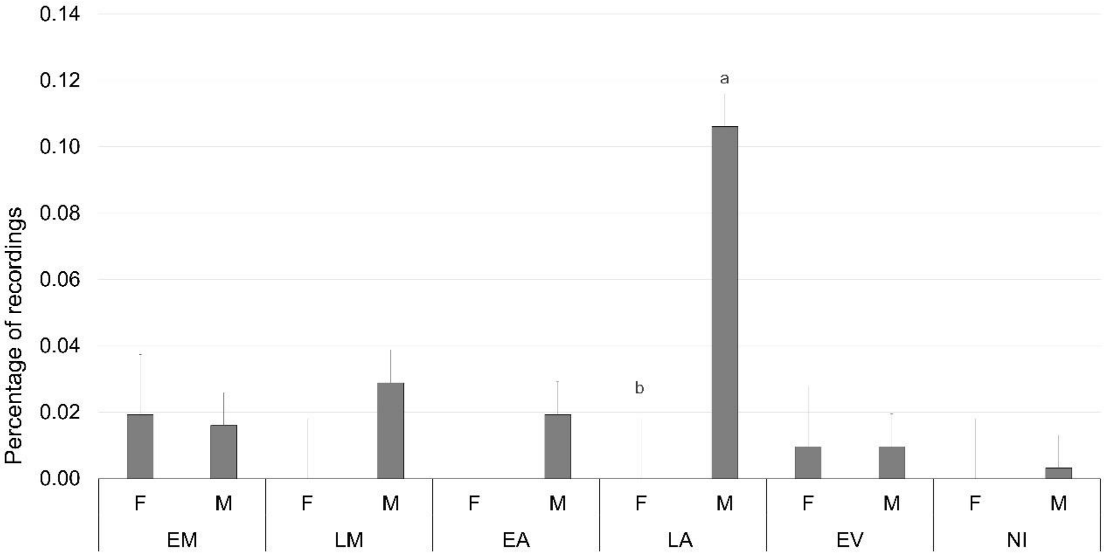

Figure 7. Least square means of the interaction of diet (CTR, 0%of HSC; T3, 3%of HSC; T6, 6% of HSC), and timing (EM, early morning; LM, late morning; EA, early afternoon; LA, late afternoon; EV, evening; NI, night) on positive interaction behavior expressed as a percentage of recordings. Black lines represent SE. Different letter differ statistically (P < 0.05)

5.5 Discussion

As hemp has been a controversial ingredient due to its THC and CBD content, its use as an ingredient in calves' diets should not change the behavior the animals normally show when they are fed with conventional diets. Resting is the most frequently seen behavior in veal calves in the literature (11), and this study supports this fact. The amount of time spent on resting depends, above all, on the space allowance (12). According to EU regulations, housed calves should have the possibility of lying simultaneously with a minimum space allowance of 1.5 m2 for each calf of a live weight of <150 kg (Council Directive 98/58/EC). In this trial, the space for each animal was 1.9 m2, so this behavior took place under normal standards. Lateral recumbency is considered an abnormal posture when it lasts for long periods (13). Positively, in this trial, lateral recumbency was seen less than sternal recumbency. Furthermore, it was observed that recumbency increased steadily as day turned into night. This could be explained by the literature since this inactive behavior was found to be more common at night than during the day (11). Rumination was the second most seen behavior in veal calves, and it is known that it allows the use of solid feed and may be affected by the type of feed available (14). Not considering the early morning and late afternoon, the amount of time spent doing cross-sucking in the other time slots was less than the normal percentage of 0.5 (15). This may be due to the fact that group housing normally increases this behavioral disturbance, even though this type of housing system is beneficial for the calves' welfare (16). However, the fact that this behavior was noticed most after the evening meal is normal because cross-sucking occurs strongly within 10– 15 min after milk feeding (16). Stereotypical behaviors in cattle have been generally highlighted more in traditional tie-stalls than in loose-housing systems, and their expression also seems to increase when restricted feed is provided (17). The overall small number of stereotypies observed in this study is likely to depend on the situation of loose housing despite the restricted feeding typical of rearing systems for fattening calves.

Regarding the more time spent eating solids in the late morning by the CTR group and knowing that the solid feed was given right after the liquid feed, it may be that the HSC inclusion in the milk somehow increased the appetite of the calves; thus, they ate the solid feed faster than the CTR group. Considering this hypothesis, HSC may also increase feed intake when offered ad libitum, but the only study to date that used 3% of HSC in the concentrate of Holstein veal calves did not find any increase in feed intake (18). HSC inclusion in females' diets made them more active, whereas males did not follow the same pattern, maybe due to a hormone interaction. The effect of the diet diminishing the duration of males doing cross-sucking is positive since it is a non-nutritive behavior that normally disappears when the calves are weaned (16). Even though sexual behavior is one of the least seen behaviors, it is important to discuss it because it is a big part of the calves' life that develops around the age range of 4–6 months (19). It was normal that the calves in this experiment expressed this behavior because they reached 6 months of age at the end of the trial. In addition, these results agree with the study of van Ek (19), who reported 0.18% of time spent in this behavior by calves of 4–6 months of age and also found that bull calves had more sexual activity than females and that they displayed more sexual interaction during the morning (07.00 a.m.−11.00 a.m.) and the afternoon (4.00 p.m.−7.00 p.m.) (19).

5.6 Conclusions

In conclusion, this study demonstrated that hemp seed cake had little effect on calves' behavior and that calves, in general, spend most of their time resting and ruminating, as they normally do with conventional diets. HSC inclusion increased the appetite for solid food and licking behavior during the late afternoon. The highest hempseed inclusion increased the female calves' movement in the late afternoon. Male calves decreased their positive interaction, movement, and cross-sucking in the late afternoon as the inclusion of HSC increased. Considering the findings given above, the inclusion of hempseed cake into veal calves' diet can be suggested, but further studies on different breeds and individual ages and the relationship between the cannabinoid content of hemp would be interesting for a better understanding of this novel ingredient.

5.7 References

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Chapter 6.

Physical Characterization of Ten Hemp Varieties to Use as Animal Bedding Material

6.1 Abstract

Hemp (*Cannabis sativa* L.) hurds, the inner bark of the stem, are a poorly appreciated part of the plant that typically represents waste. The aim of this experiment was to describe the physical characteristics, including moisture (M), water absorption (WA), and ammonia absorption (AA), of 10 hemp varieties (Fibranova, Codimono, USO31, CS, Futura 75, Eletta Campana, Carmaleonte, Felina 32, Santhica, and Ferimon) cultivated in Italy. Samples of hemp hurds were ground to 8 mm obtaining hemp shives. Values of M, WA, and AA were determined following the official procedures. The results showed an average of 7.78%, 251.9%, and 50.0% for M, WA, and AA, respectively. Data of M and WA were similar among varieties, whereas a significant difference was found for the AA, varying from 45.0 to 55.5% for the Fibranova and Ferimon varieties, respectively. In conclusion, hemp shives have good physical characteristics, similar to other commercial bedding materials (i.e., wood shavings) but other parameters and on-farm trials will be required to make a full assessment of hemp.

6.2 Introduction

Several materials have been used for livestock bedding. A good bedding material should provide animal comfort, have good absorption capacity of water and ammonia, decompose quickly with manure, be economic and not cause hygiene problems [1–5]. Among the popular bedding materials are crop straws, wood shavings, peat, seed hulls, and corn stover [2,6–10]. Crop straws are the fibrous residue from grain crop harvest and may be one of the most common bedding materials used in farms of Central Europe and southern Nordics countries [1,3]. Wood shavings are used worldwide, mostly because it is one of the cheapest options in the market [1,4]. Peat is a quite common bedding material, especially in countries like Finland and Sweden [1]. Seed hulls, mostly represented by rice hulls is an important by-product of the rice milling process. Corn stover, which is a by-product of the processing of the most important cereal of the world [3]. Even though we have all these well know bedding materials, some other crop residues are always being tested as a simple way to valorize agro-waste resources [11] because they are continuously generated in large quantities all over the world [12].

Hemp (*Cannabis sativa* L.), an ancient plant primarily cultivated for its fiber, has great added value because each part of the plant represents many potentially valuable resources for quality products [13]. As a muti-purpose crop, hemp delivers: fibre, hurds, seeds, flowers and leaves. Fibre is used for the obtention of paper, biocomposites and insulation materials. Hurds are used for construction and animal bedding. Seeds have a high nutritional value with an excellent and unique fatty acid profile so it is used to produce oil and other by-products. Flowers and leaves processing lead to the obtention of pharmaceutical and food supplements that contain non-psychotropic cannabinoid (CBD) and it is used for medical purposes [14]. The diversity of hemp is shown by the 70 varieties included in the EU Common Catalogue of Varieties of Agricultural Plant Species that can be divided into two broad categories, those suited for seed production and those for fiber production. Among the seed varieties, Felina 32 and Ferimon are included. Whereas, CS, Fibranova, Eletta Campana, Futura 75 and Santhica are considered fiber varieties. They are generally taller (1–5 m) [12], and exhibit optimum fiber yields when cultivated in temperate climates with an annual rainfall on average of 630–750 mm [15]. In Italy, hemp is sown in spring (April–May) and harvested in autumn (September–October). The harvesting of plants for fiber production (roughly 70–90 days after sowing) is preferably made at the flowering stage, as further maturation increases the proportion of undesirable “secondary” bast fibers in plants [15]. Whether hemp’s main purpose is to obtain seeds or fiber, hemp hurds always end up as a sub-product [16]. In fact, the relation between hemp hurds and fibers is 1.7 to 1 [16]. Hemp hurds are basically the inner bark of the stem, which is the hemp core or the leftover bast fiber that typically contains around 20 to 30% of lignin. They are a poorly appreciated part of the plant which typically ends up as landfill [17], embedded in the ground or, more recently, if collected, used in green buildings [12].

As hemp production is increasing in Europe, along with the global need to guarantee sustainable crop management using zero-waste strategies, the evaluation of using hemp hurds as a potential bedding material is needed. At present, hemp hurds are considered one of the most interesting waste products obtained from hemp [16]. As they can absorb moisture up to four times their dry weight, they have already reached 63% of market participation as an animal bedding material for horses and other farm animals like chickens [14]. However, no studies are available on the physical properties of hemp, such as moisture content, water absorption, or ammonia absorption to increase its use in this field. Therefore, this study evaluated 10 different hemp varieties cultivated in Italy in order to verify the main physical parameters and define their viability for livestock bedding.

6.3 Materials and Methods

6.3.1 Test Materials

Ten varieties of *Cannabis sativa* L. were evaluated: Fibranova, Codimono, USO31, CS, Futura 75, Eletta Campana, Carmaleonte, Felina 32, Santhica, and Ferimon. They were cultivated at the Center for Cereal and Industrial Crops (CREA-CI), located in Rovigo (Veneto Region, Northern Italy).

6.3.2 Hemp Sample Obtention

After harvesting, 30 plants were randomly selected from each variety. The separation of the fiber was carried out at CREA-CI (Rovigo, Italy) and the final step for the sample obtention was made at the Bio-fuel Analysis Laboratory (ABC Laboratory) of the Department of Land, Environment, Agriculture and Forestry (TESAF) of the University of Padua.

The central part of the stem was cut to obtain 60 cm stalks that were left in the sun to dry naturally. The stalks were totally immersed under water and remained at a temperature of 30–35

°C for 7 days. Then, each stalk was rinsed with clean water until the mucilage was completely removed and the fiber separated (Figure 1a).

After this, they were dried in the sun for 24 h and then dried in an oven at 60 °C for 3 days until a constant weight was obtained. Finally, they were processed using a cutting mill (SM 100 RETSCH GmbH, Haan, Germany) equipped with an 8 mm sieve in order to obtain the hemp shives (Figure 1b).

(a) **(b)**

Figure 1. Hemp sample treatments, (a) fibre removal; (b) hemp shives.

6.3.3 Physical Measurements

Analyses were carried out in the ABC Laboratory, where moisture content (M), water absorption (WA), and ammonia absorption (AA) were analyzed. For the moisture content, three repetitions of each variety were measured following the standard procedure of UNI EN ISO 18134-1 (2015). A 300 g sample was oven dried at 105 _C. Every 60 min, the samples were taken from the oven and weighed until two consecutive weights were found to be stable (with under 0.2% variation allowed). Then, M was calculated from the difference in the sample weight before and after drying and expressed as a percentage. The WA was assessed following the procedure of Potgieter and Wilke (1996) [18], in which, 150 mL of water and 10 g of sample were left to soak for one hour in a closed filter funnel. Then, the funnel was opened to drain the excess water through filtration. The AA followed the procedure of Fleming et al. (2008) [19], but ammonia 9% was used instead of the mixture of horse urine and feces.

6.3.4 Statistical Measurements

Statistical analysis was conducted using PROC GLM procedure in SAS (SAS Institute Inc., Cary, NC, USA, 2009). Data from M, WA, and AA were analyzed with a monofactorial model that considered the effect of variety (10 levels). For all the variables, the comparisons between LS means were performed using the Tukey test, and differences were considered significant at $p < 0.05$.

6.4 Results

6.4.1 Moisture Content

All the values of moisture content were similar ($p > 0.05$) among varieties (Table 1). The mean value was 7.78 ± 0.29%.

6.4.2 Water Absorption

No statistical differences ($p > 0.05$) were observed among varieties (Table 1), even if the range between the highest value (317.9%; Felina 32) and the lowest value (211.4%; Eletta Campana) was very wide.

6.4.3 Ammonia Absorption

The values of ammonia absorption (Table 1) ranged from 45.0% (Fibranova) to 55.5% (Ferimon). The differences among varieties were statistically significant ($p < 0.05$).

Table 1. LS means of physical properties of hemp shives.

Variety	M	WA	AA
Fibranova	7.97	250.4	45.0
Codimono	7.70	212.3	53.2
USO31	8.21	281.5	51.7
CS	7.70	235.1	51.0
Futura 75	7.73	235.4	49.1
E. Campana	7.72	211.4	47.6
Carmaleonte	7.64	310.0	49.7
Felina 32	7.52	317.9	48.9
Santhica	7.69	234.2	48.6
Ferimon	7.91	231.1	55.5
p-value	0.5946	0.2001	0.0452

M: moisture content; WA: water absorption; AA: ammonia absorption.

6.5 Discussion

As no previous study of hemp as material for animal bedding has been carried out before, the whole method of processing the hemp to obtain the samples and the methods used to analyze the physical characteristics were difficult to choose but are essential to understand and explain our results. Hemp hurds were chosen because the hemp fiber industry obtains dust-free hemp hurds as a waste product that can be directly used for livestock bedding. Indeed, there is already a stable market for this commodity, mostly for pets and horses [20]. Starting from the decortication process, which is the separation of fibers from hurds, we can already point out some aspects to consider. The type of process applied for hemp fiber extraction has an effect on the chemical composition of the fibers and the resulting properties not only of the fibers themselves but also of the hurds obtained as a co-product. For this experiment, the method of fiber extraction was similar to that called osmotic degumming [21]. Of course, the different methods of fiber extraction made on a large scale by hemp factories will change the quality of hurds and their properties considerably [21]. After obtaining the hurds, a further step was needed to transform them into an appealing bedding material. In order to do that, we used a mill to turn the hemp hurds into hemp shives. It is clear that the particle size obtained after the whole processing of the hurds influenced all the physical parameters reported in this study. For instance, small particles usually give better performance for water absorption, because of the increased ratio of surface to volume [22].

Our results showed that the moisture content between hemp varieties was not significantly different. This is mostly because they come from the same field and been under the same storage conditions before the beginning of the experiment. Moisture content is an important factor to consider in the choice of any bedding material. High moisture in the bedding increases ammonia build-up through increased microbial metabolism, resulting in respiratory lesions [18], whereas a low moisture content assures a longer storage period of the bedding material since it affects the litter's physical and handling properties such as compressibility, compaction, and cohesion [23]. Hemp shives (7.78%) showed similar moisture content to other bedding materials such as wood shavings (7.1 and 7.37%), corn stover (8.06%), rice hulls (8.37, 8.7, 10%), and wheat straw (8.44%) [23–26], but higher moisture content than recycled paper (3.82%), rice husks (4.62%), and sawdust (4.83%) [22].

Water absorption is an important property of bedding material as it shows the quantity of water that the material is capable of absorbing and storing. Similar results for water absorption for the ten hemp varieties were found in this study. Literature assures that hemp hurds can absorb up to five times their weight in moisture which is typically 50% higher than wood shavings [27]. Even though it was difficult to make direct comparisons with previous reports due to the different methods used and the nature of the sample, the water absorption of hemp shives in this study was lower than the only value (325.0%) found in the literature [28]. In addition, another study reported the water absorption in hemp hurds to be 356.2%. Unfortunately, we do not have any information about the nature of either of these samples to offer any further discussion. In previous studies, water absorption of bedding materials was reported and showed values of 266% for fine wood shavings, 305% for cereal straw, 320.8% for wheat straw, 330% for straw, 382% for recycled paper, 392.3% for paper cuttings, 315.9% and 460% for wood shavings, 462% for rice husks and 483% for sawdust [1,19,22,28]. This suggests that hemp shives may have a similar water absorption capacity to fine wood shavings which are known for their good moisture absorption. High water absorption is a desirable physical characteristic because it leads to the absorption of water in excreta and urine. Water absorption of hemp could even be improved if the processing method changes, for example, by decreasing the particle size. Even though the capacity to absorb water is an important value, it differs from the capacity to absorb urine, which could be higher or lower depending on the bedding material [1].

As happened with water absorption, ammonia absorption was also a difficult parameter to compare within the literature because of differences in methods. A high concentration of ammonia inside the animal house could represent a potential health hazard to humans and animals [29], so it is better that the bedding material has a good capacity of ammonia absorption to avoid compromising the animal health status. A significant difference was found between varieties, with Ferimon showing the highest ammonia absorption. Our results were close to those of Airaksinen et al., who reported that the relative ammonia absorption of hemp was 60%, and stated that hemp had a better ammonia absorption capacity than wood shavings (44%), and straw (4%) [8]. However, that study used horse urine in its procedure and the nature of the hemp sample was not described. Ammonia absorption is important both in summer when the indoor temperature in the animal house rises, and in winter, when the ventilation has to be reduced because of the cold. Knowing that ammonia emissions coming from animal manure is a great source of atmospheric ammonia [30], this parameter could be improved by raising the water content, shredding, or the addition of an ammonia absorbent such as sodium bisulfate [1].

Considering that this study shows a general overview of hemp hurds in the form of shives as bedding material, it is clear that some other parameters still need to be determined to provide a complete assessment of this new and still little-known product. More physical parameters such as particle size, bulk density, and water-holding capacity need to be covered. As animal health and welfare are important to consider too, microbial quality, dustiness, and more ammonia tests should be performed. The availability and price of hemp hurds also need to be studied. For this reason, the amount of bedding use per animal per day, and an economic study would be useful. In Italy, commercial brands of pure hemp shavings are sold at a range of prices from 1.4 to 3.8 euros per kilogram, depending on the quantity of the material and the target animal. Those for pets like rabbits and other rodents are always more expensive than those for horses or larger animals. Finally, to complete the zero-waste cycle of hemp, the possibility of making compost or other bio-fuel products after the farm cycle finishes needs further investigation.

6.6 Conclusions

In conclusion, the physical characteristics of hemp shives give them the ability to become a good animal bedding material. Any of the ten hemp varieties studied could be used, alone or as a mixture, as there was no wide variation among them apart from the ammonia absorption. In addition, a comparison with other studies indicates that hemp has similar water and ammonia absorption capacities to other commercial bedding materials like wood shavings. In the future, the impact of hemp will need to be evaluated through on-farm trials.

6.7 References

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General conclusions

The exploration of industrial hemp (*Cannabis sativa* L.) and its derivatives (cake, meal, and oil) has been updated through some studies that involved firstly the chemical and nutritional characteristics of different varieties of hemp and products, to then testing its effect as feed for ruminants. This investigation sheds light on crucial aspects that contribute to the potential of industrial hemp as a highly beneficial food or feed source.

1. Nutritional Composition:

    - The proportion of botanical fractions and the chemical composition of industrial hemp is strongly influenced by the agro-climatic conditions.
    - Hemp and products exhibit good amounts of fat and protein. They contain high amounts of polyunsaturated fatty acids, primarily linoleic and α-linolenic acid. The amino acid profile showed that arginine and glutamic acid were the most abundant amino acids. Moreover, hemp constitutes a good source of essential amino acids because it fulfils almost 20% of the daily requirement in humans. In addition, the mineral content showed high levels of manganese which can completely fulfill and exceed the nutrient reference values in humans.
    - From all hemp parts and products, hemp cake, showed its great nutritional potential as a valuable food or feed ingredient. Seeds and leaves, are also viable protein sources, emphasizing the versatility of hemp in various applications.
    - Cannabinoid content, specifically THC, is present at very low values in industrial hemp, suggesting none or minimal psychoactive impact.

2. Varietal Differences:

    - Varietal differences in all nutrients were observed. CS, Eletta Campana and Tisza were identified as promising varieties due to their high protein and fat content.

3. Ruminant Diet Inclusion:

    - Dosages of the hemp by-products used in all the in-vivo trials of this thesis respected the stablished by European Food Safety Authority (EFSA) and confirmed that those levels were safe for the animals. Literature stated that hemp oil's fatty acid composition facilitates the transfer of polyunsaturated fatty acids into the milk of dairy ruminants. Unfortunately, the same type of enrichment in meat using hemp cake was not successful because it possibly requires higher doses.
    - Inclusion of hemp cake in Holstein veal calves' concentrate is considered safe, with no negative effects on health or in vivo performance parameters. Moreover, meat color remained pale so it will still be well accepted by consumers.

- Hemp seed cake inclusion diluted in the milk replacer of Holstein veal calves did not change their natural behavior. Some minimal effect was seen mostly in the late afternoon as they increased their appetite for solid food and licking behavior. Also, the highest hempseed inclusion increased the female calves' movement. Male calves decreased their positive interaction, movement, and cross-sucking as the inclusion increased.

4. Other uses in animals

   - Hemp shives, another hemp by-product from the stem of the plant, are suitable as animal bedding material because they display good physical characteristics such as high water and ammonia absorption capacity.

5. Future Research and Development:

   - Research on hemp varieties in other countries with different climate conditions are essential for designing high-quality products and aiding breeders in improving agronomic aspects based on nutritional values.
   - More studies are needed to quantify the presence of cannabinoids, antinutritional compounds, phenolic compounds and bioactive peptides. To then assess the effect on animal behavior, performance and production. Considering also, the cumulative effect that might occur with the continuous supply of cannabinoids.
   - The impact of hemp shives as bedding material will need to be evaluated through on-farm trials in order to see the animal response.

To conclude, the chemical composition of industrial hemp demonstrates that this versatile crop is a nutritionally valuable resource for feeding ruminants in a sustainable way, but further exploration for discovering its effect in animal health, behavior and performance need to be done.

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