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Review: Nutritional aspects of hemp-based products and their effects on health and performance of monogastric animals



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ABSTRACT

Sustainable agriculture aims to produce food and feed that ensure food security and play a key role in environmental protection. For this, producers, supported by scientific research, are investigating new protein alternatives for animals that guarantee high performance and preserve their health. Among these, hemp (Cannabis sativa L.) is gaining great success, both for its active role in environmental conservation and for the high nutritional profile of the seeds (20–30% carbohydrates, 25–30% proteins easy to digest and rich in essential amino acids, and 25–35% lipids with a balanced fatty acid composition), also ensured by the co-products, particularly seed cakes (30-34% proteins and 10-12% lipids). However, the last scientific report by the European Food Safety Authority for the use of hemp-based products in the feed sector now dates back to 2011. For this reason, the objective of this review, in addition to outlining the nutritional profile of hempseeds (HSs) and co-products, aims to investigate their use in the monogastric sector, particularly in the diets of pigs, broilers, and laying hens, by summarising the main works in the literature up to 2023, investigating the effects on animal health and performances. The reported results showed that the addition of 50 g/kg of HSs and HS oil improved the nutritional profile of milk and colostrum in lactating sows, particularly the lipid profile, positively affecting the health of piglets. For broilers, the inclusion of HSs (20 g/kg) resulted in better values on growth performance. This was not matched by the addition of HS oil (up to 60 g/kg). In particular, although a better polyunsaturated fatty acid profile was observed, the results on growth performance were contradictory. The same trend was observed for HSs cakes with 50, 150, and 200 g/kg inclusion. For laying hens, the inclusion of HSs (up to 250 g/ kg), HS oil (up to 300 g/kg), and HSs cake (up to 150 g/kg) increased the nutritional and functional profile of the eggs, safeguarding performance and animal welfare. However, despite the promising results, the function of hemp-based products in the diet of monogastric animals needs to be further investigated to identify the optimal level of inclusion and timing of administration, necessary to ensure high performance and health of the animals.

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Implications

Feed from sustainable agriculture is a key issue in the livestock sector. Producers, supported by research, are investigating new alternatives that are able to satisfy their principles. Hemp (*Cannabis sativa* L.) represents a viable alternative, both for its high nutritional profile and its low environmental impact. For this reason, the aim of the following review was to investigate the use of

hemp-based products in addition to the diet of monogastric animals, demonstrating the potential of these products on the health and performance of swine, broilers and laying hens.

Introduction

Sustainable agriculture is the production of agricultural products at an environmental cost that does not jeopardise food/feed security and animal health. For this reason, scientific research is investigating new alternatives to classical protein sources to ensure these principles. This objective is strongly pursued by the European Union through the One Health approach, and

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subsequently with the Green Deal, dated 1 December 2019, which recognise that human, animal, and environmental health are interconnected, promoting efficient use of resources through a circular and clean economy (Wolf et al., 2021). Among the possible alternatives, *Cannabis sativa* L, commonly known as hemp, is gaining increasing curiosity in the food/feed sector, not only due to the high nutritional and functional properties of hempseeds (**HSs**) but also for the role it plays in environmental protection. As well as being able to grow rapidly in different agro-ecological conditions (it can reach four metres in height) while requiring limited amounts of water and herbicides, it is an excellent candidate for carbon sequestration (nine to thirteen tonnes/ha). In addition, its deep roots (about two metres long) prevent soil erosion and also take up nutrients and metals from the deeper soil layers (Rupasinghe et al., 2020; Rehman et al., 2021).

In particular, interest in hemp has grown following the European Regulation 1251/1999/EC, which established a support system for all producers of particular types of seeds, including industrial hemp, and European Regulation 1370/2013, with which hemp cultivation was included among those eligible for Common Agriculture Policy payments with the only condition that the seeds used for cultivation be of varieties registered in the European catalogue with a Δ 9-tetrahydrocannabinol content of less than 0.2–0.3% (Sorrentino, 2021).

To ensure sustainable agriculture, one possible approach involves recycling nutrients from residues and unusable crop coproducts and adding them to the diet of livestock animals, among these, hemp co-products are attracting great interest, in particular the whole plant, leaves and flowers (obtained before/after the cannabinoid extraction process), stems, stalks, whole seed heads, and seed hulls, as reported by Kleinhenz et al. (2020), Vastolo et al. (2022), and Ely and Fike (2022).

However, the last report by the European Food Safety Authority (**EFSA**) on the use of hemp-based products in feed sector, entitled *Scientific Opinion on the safety on hemp (Cannabis genus) for use as animal feed*, dates back to 2011 (EFSA, 2011). More precisely, in this report, EFSA suggests inclusion levels based on the work published until then. As might be expected, the number of scientific publications investigating the use of hemp-based products in the diets of monogastric animals has increased since 2011.

For this reason, the objective of the following review, in addition to outlining the nutritional profile of HSs and co-products, aims to investigate their use in the monogastric sector (pigs, broilers, and laying hens) by summarising the main works in the literature up to 2023, investigating the effects on animal health and performances.

Nutritional properties of hempseeds

Hempseed is the main component of the plant used in the food and feed sector due to its very promising nutritional and functional properties. It is an achene, enveloped by a thin and hard pericarp characterised by a high amount of fibre, in particular 40–50% NDF, 30–35% ADF, and 10–15% ADL, 23–30% proteins easy to digest and rich in essential amino acids (**AAs**), 25–35% lipids with balanced fatty acids and 5% of ashes (Farinon et al., 2020; Fike, 2019; Lanzoni et al., 2023). These values, as shown in Table 1, are similar to those of flaxseeds, an important matrix in the food/ feed industry.

However, as reported in the literature, these percentages depend on the plant genotype, environmental growth factors and, above all, the type of technological treatment the seed is subjected (Fike, 2019; Leonard et al., 2020; Lanzoni et al., 2023). The fibrous fraction of HSs is characterised by a soluble:insoluble fibre ratio of 20:80, a similar value to that of other food/feed sources, such as flaxseeds (Callaway, 2004; Farinon et al., 2020). However, due to treatments such as heating and extrusion, it is possible to concentrate fibre fraction, making them more appreciable for food and feed applications (Amaducci et al., 2008).

In HSs, the proteins are mostly located in the inner layer of the seed, with a low fraction in the hull. To date 181 proteins have been identified, the most common of which are edestin, albumin, and β -conglycinin (Farinon et al., 2020). Edestin accounts for approximately 60-80% of the total protein content, albumin accounts for approximately 25%, and β-conglycinin accounts for approximately 5% (Farinon et al., 2020; Odani and Odani, 1998). Edestin, whose molecular weight is 300 kDa, is made up of six identical subunits, each of which reorganises itself into an acidic and a basic subunit, held together by sulphur bonds, which make it less flexible than albumin. However, the main difference between these two proteins is their AA composition. Edestin is characterised by a greater number of aromatic, branched-chain, and above all, sulphur-rich AAs such as cysteine and methionine, which provide greater nutritional value than albumin (Wang and Xiong, 2019). In general, HSs provide a qualitatively and quantitatively high AA profile, enriched by the presence of essential AAs, resulting in a high biological value, although lysine, cysteine and tryptophan are limiting AAs. However, as demonstrated by Callaway (2004), the AA composition is comparable to that of other high-quality proteins, such as soybeans and rapeseeds.

The lipid content of HSs, called also oil, is characterised by a valuable nutritional profile, as reported in Table 2.

It is distinguished by a low content of saturated fatty acids (SFAs) $(10.9 \pm 0.7\%)$ and a high content of unsaturated fatty acids. in particular polyunsaturated fatty acids (**PUFAs**). The latter, as shown in Table 2, accounts for about 75.1 ± 3.7% of the entire oil fraction, reaching up to 84% (Callaway, 2004; Siano et al., 2018). Of the PUFAs present, the most prevalent are linoleic acid (18:2, n-6) and α -linoleic acid (18:3, n-3, **ALA**), defined as essential fatty acids. They are the precursors of long-chain PUFAs, arachidonic acid (20:4, n-6), docosahexaenoic acid (22:6, n-3), and eicosapentaenoic acid (20:5, n-3), which play key roles in brain development, cardiovascular health, obesity, and in the regulation of metabolic and inflammatory processes, via the synthesis of prostaglandins and leukotrienes (Farinon et al., 2020). High levels of linoleic acid and ALA present within the lipid component of HSs mean that the n-6/n-3 ratio, responsible for physical well-being, is low, approximately 3.5, defined by the EFSA as the ideal intake (Leonard et al., 2020; EFSA, 2009). In addition to linoleic acid and ALA, HS oil also contains their respective biological metabolites y-linolenic Acid (18:3, n-6) and stearidonic acid (18:4, n-3), which allow the first critical enzymatic step of δ -6-desaturase to be bypassed, facilitating conversion into the biologically active form of long-chain PUFAs (Farinon et al., 2020). Among the monounsaturated fatty acids

Table 1

Nutritional profile of hempseeds compared to flaxseeds (% w/w on DM basis) used in the monogastric feed sector. Data are presented as mean ± SEM. EE = ether extract. Table adapted from Lanzoni et al. (2023).

Samples	DM	СР	EE	NDF	ADF	ADL	ASHES
Hempseeds	94.6 ± 0.12	23.1 ± 0.57	27.9 ± 0.75	44.6 ± 0.21	33.2 ± 0.31	14.4 ± 0.32	5.8 ± 0.10
Flaxseeds	91.6 ± 0.14	23.0 ± 0.45	36.0 ± 0.07	41.5 ± 1.27	19.5 ± 0.35	9.2 ± 0.41	2.7 ± 0.20

Fatty acid profile of hempseeds used in the monogastric feed sector. Values are expressed in % of oil. When several cultivars were analysed, the maximum and minimum values of the range of data obtained are indicated. When only one cultivar was analysed, the mean ± SD is reported. PA: Palmitic acid; SA: Stearic acid; OA: Oleic acid; LA: Linoleic acid; GLA: γ-Linolenic acid; ALA: α-Linolenic acid; SDA: Stearidonic acid; SFAs: saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; n.a.: not available. Table reported by Farinon et al., 2020.

PA	SA	OA	LA	GLA	ALA	SDA	SFAs	MUFAs	PUFAs	n-6/n-3	REF
5.0	2.0	9.0	56.0	4.0	22.0	2	n.a.	n.a.	84.0	2.5	Callaway, 2004
7.0 ± 0.3	2.8 ± 0.4	12.7 ± 1.3	56.2 ± 3.5	2.9 ± 0.4	15.0 ± 1.1	n.a.	10.9 ± 0.5	13.1 ± 1.3	75.1 ± 3.7	4	Siano et al., 2018
5.6 ± 0.5	3.9 ± 0.4	16.2 ± 6.2	54.7 ± 4.1	n.a.	16.2 ± 4.0	0.5 ± 0.01	10.9 ± 0.7	17.5 ± 6.3	72 ± 4.3	3.2	Vecka et al., 2019
6.7-7.0	2.1-2.8	9.4-13.0	55.6-56.6	2.6-4.5	14.7-17.3	n.a.	9.6-10.3	n.a.	n.a.	n.a.	Vonapartis et al., 2015
6.7-7.3	2.3-3.5	9.2-15.7	55.0-58.2	0.6-4.5	12.6-19.6	0.2-1.5	9.4-11.7	9.7-16.1	72.2-80.7	2.8-4.5	Galasso et al., 2016
7.1-9.1	2.1-2.8	10.3-17.9	51.6-54.2	1.9-5.0	10.5-15.3	n.a.	n.a.	n.a.	n.a.	3.9-5.5	Irakli et al., 2019
6.1–7.8	2.3-4.0	12.2-18.8	53.9-59.0	3.5-6.2	12.3-18.9	n.a.	n.a.	n.a.	72.0-78.6	3.2-5.0	Lan et al., 2019

(**MUFAs**), the most prevalent is oleic acid (18:1, n-9) which is present in quantities comparable to flaxseeds and greater than chia seeds (Montserrat-de la Paz et al., 2014).

Although, as previously reported, HSs are characterised by a high nutritional profile, it is important to underline the presence of anti-nutritional factors as well. The main anti-nutritional factors in HSs are phytic acid, trypsin inhibitors, condensed tannins, saponins, and non-starch polysaccharides. Although phytic acid plays an important role in preventing lipid peroxidation, it can alter the activity of digestive enzymes, impairing the bioavailability of minerals and proteins (Russo and Reggiani, 2015). The phytic acid content in HSs is found to be around 4-8% of the dry weight of defatted matter, a higher concentration than that found in soybeans (2%) (Russo, 2013; Russo and Reggiani, 2015;). Condensed tannins are phenolic compounds and therefore have a high antioxidant, anti-inflammatory, and anti-microbial activity. However, they are considered to be anti-nutritional factors as they form insoluble complexes with minerals and proteins, thus affecting their absorption by the body (Mattila et al., 2018). Although, within HSs, trypsin inhibitors are present in a lower concentration than in soybeans, they play a negative role during the digestive process. In particular, as reported by Farinon et al. (2020), these antinutritional factors inhibit the action of proteinases in the stomach and trypsin and chymotrypsin in the intestine, not allowing the degradation of protein chains, thus reducing AA bioavailability. Saponins are able to cause gastritis and haemolysis of red blood cells, only when ingested in high quantities. However, the concentration of these anti-nutritional factors is lower than in common matrices such as flaxseeds, quinoa seeds and soybeans (Russo and Reggiani, 2015). Finally, HSs are characterised by the presence of non-starch polysaccharides, albeit little explored in the literature. As reported by Hetland et al. (2004), non-starch polysaccharides are compounds that can resist the animal's digestive enzymes, creating a highly viscous intestinal environment, consequently affecting the digestion and absorption of nutrients. As reported by House et al. (2010), the main non-starch polysaccharides in HSs are Rhamnose $(1.14 \pm 0.17 \text{ mg/g})$, Arabinose $(2.99 \pm 0.17 \text{ mg/g})$ 32 mg/g), Xylose (74.74 ± 15.74 mg/g), Mannose (1.23 ± 0.21 mg/ g), Galactose (2.61 ± 0.28 mg/g) and Glucose (76.52 ± 19.84 mg/g). Although these values are elevated, processes involving HSs such as dehulling can drastically reduce these values, emphasising that non-starch polysaccharides are mainly present in the seed hull (House et al., 2010). In light of the above, although HSs are characterised by the presence of anti-nutritional factors, their content is not only highly comparable to that of other matrices used in the feed industry but can also be modulated with treatments as observed for non-starch polysaccharides, consequently increasing the nutritional profile.

Nutritional properties of hemp co-products

The use of co-products is a valid strategy to ensure the sustainability of the feed sector. As reported above and shown in Table 3, the main hemp co-products investigated in the scientific literature are whole plant, leaves, flowers, stems, stalks, seeds cake, chaffs, seed heads and hulls.

The percentages of these nutrients vary depending on the cultivation techniques, environmental conditions, and genotype analysed.

In particular, the CP content varies from 5.4 to 34.4%, with higher values reported for HS cake (30–34.4%), due to the high level of the protein content following the oil extraction process. At the same time, as reported by Vastolo et al. (2022), this product has a high protein digestibility, tested following the in vitro digestion process, showing values highly comparable to tobacco coproducts. Interesting values include seed heads (23%), chaffs (21.2%), hulls (21.9%), and exhausted biomasses (Kleinhenz et al., 2020; Vastolo et al., 2022; Ely and Fike, 2022). As reported by Ates (2021), the total protein content of co-products, obtained as a result of the cannabinoid extraction process, is similar to alfalfa, with higher values for the lipid content.

As shown in Table 3, hemp co-products are characterised also by high level of fibre. The NDF ranges from 23.4 to 84.4%, which is lower for co-products rich in leaf and flower components. The latter have higher digestibility than corn stalks, oat or barley straw. However, as reported by Kleinhenz et al. (2020), such co-products are poor sources of energy within livestock diets, suggesting a different use as a source of dietary fibre, especially in ruminants.

Furthermore, as reported by Ely and Fike (2022), being an annual crop that grows when most cool-season fodder slows down, hemp could have potential as an alternative summer fodder or used in emergencies, although to date, there are few studies available that evaluate its use as fodder. However, at the same time, hemp co-products have some limitations. These include the high variability of nutrients due to different processing methods and the need for essential preservation treatments to stabilise the product, especially those with high moisture and lipid values (Ely and Fike, 2022).

Use of hemp-based products in monogastric animals

Swine

The use of HSs and hemp-based products in the swine sector is still at an early stage. As shown in Table 4, the main studies concern the use of these products in diets for lactating sows and piglets, which are key steps for the success of the entire production cycle. Weaning piglets represent the most delicate and crucial time for the health of these animals. During this phase, the animals face environmental (separation from the mother), social (union of different broods), and nutritional (transition from mother's milk to solid feed) changes. All these factors act as stressors, simultaneously causing both a decrease in feed intake (**FI**) and the development of gastric and intestinal problems, resulting in severe physiological and immunological disorders (Palade et al., 2019; Vodolazska and Lauridsen, 2020). For this reason, it is necessary

Chemical Composition of hemp co-products (whole plant, leaves, stalk, seed cake, seed heads, chaff, extracted flower, whole plant SHB, stem and leaves SHB, hemp hulls) used in the monogastric feed sector. Values are expressed in % DM. Source: Kleinhenz et al. (2020), Vastolo et al. (2022) and Ely and Fike (2022). SHB: Spent hemp biomass.

Item	Whole Plant	Leaves	Stalks	Seeds Cake	Seeds heads	Chaffs	Extracted flowers	Whole plant SHB	Stems & Leaves SHB	Hemp hulls
СР	6.9	13.0	5.3	30.0-34.4	23.0	21.2	24.5	22.4	19.2	21.9
FAT	2.7	8.9	1.2	10.2-12.4	13.2	4.6	3.2	4.3	7.5	23.5
ADF	60.8	20.8	64.6	32.1-39.5	29.6	18.0	18.1	32.3	17.6	37.9
NDF	81.6	44.7	84.4	39.3-51.9	53.2	27.9	30.9	40.1	23.4	57.1

Table 4

Effects of the inclusion of hemp-based products (HSs, HS oil, HS meal) in the swine (sows and piglets) diet. HSs: Hempseeds; FA: Fatty acids.

Total number of animals	Experimental Period	Treatments	Effects	Ref
10 sows and 96 suckling piglets	21 days	 Control group; Sows (50 g/kg of HSs); Piglets (15 g/kg of HSs). 	Increased n-3 FAs profile; Decreased n-6/n-3 in colostrum and milk; Increased Milk Yield; Increased Average Daily Gain during first week.	Habeanu et al., 2018
24 sows	35 days	 Control group; 50 g/kg HS oil; 25 g/kg HS oil + 25 g/kg soybean oil). 	Increased n-3 FAs profile; Decreased n-6/n-3 in colostrum and milk; Decreased n-6/n-3 in plasma of sows and piglets; Decreased sows final BW; Increased of total and live-born piglets; Increased weight gain of piglets during first week.	Vodolazska and Lauridsen, 2020
7 sows	28 days	Control group;16 g/kg of HS oil.	No differences in live-born piglets; Increased final BW of piglets; Increased Average Daily Gain; Increased sows final BW.	Idricenau et al., 2021
10 sows and 16 suckling piglets	31 days	 Control group; Sows (20 g/kg of HS meal before farrowing; 50 g/kg of HSs throughout the lactation period). Piglets (15 g/kg of HS meal). 	Increased anti-oxidative status of sows; Increased anti-oxidative status of piglets.	Palade et al., 2019

that the energy and nutritional intake provided through breast milk is high, to overcome this critical phase. Although piglets are born with an energy deficit and low-fat reserves, the absorption of lipids and fatty acids from colostrum and milk is efficient (Palade et al., 2019; Vodolazska and Lauridsen, 2020). For this, animal producers supported by research are trying to intervene through diet to safeguard their health. One of the possible alternatives, still little explored in the literature, concerns the use of hemp-based products in sow rations to study how piglets' performance varies.

As shown in Table 4, the addition of HSs (up to 50 g/kg) in the diets of lactating sows resulted in positive results on the growth performance of piglets. This result, as reported by Habeanu et al. (2018), is related both to the direct consequence of lipid supplementation in the diet, which allows for an increase in milk fat and thus a higher energy intake, and to the high nutritional profile, especially the lipid content of HSs. Indeed, Habeanu et al. (2018) observed that the addition of HSs increased the n-3 PUFAs content in the milk of treated sows (6.66% of total content) compared to the control group (soybean meal) (4.35%), which resulted in a lower n-6/n-3 ratio (6.91 vs 10.52, respectively). Similarly, positive results were obtained with the inclusion of 50 g/kg HS oil in the diet (Vodolazska and Lauridsen, 2020). Vodolazska and Lauridsen (2020) found high concentrations of ALA (C18:3, n-3), arachidonic acid (C20:4, n-6), and stearidonic acid (C18:4, n-3), resulting in an even lower n-6/n-3 ratio (4.30) in the milk of treated sows. Positive growth performance was also observed with a lower (16 g/kg) inclusion level of HS oil (Idricenau et al., 2021).

As previously reported, in the pig sector, particular attention is focused on the weaning period. However, at this stage, according to the literature, sows can lose an average of 15–40 kg during the entire lactation period (Hansen, 2012). For this reason, it is neces-

sary to select a diet that guarantees both the health of the piglets and that of the sows.

Although, as previously shown, the addition of two different concentrations of HS oil resulted in the same positive effects on piglet performance, the inclusion of 50 g/kg HS oil affected the total weight of the lactating sows (320 ± 0.18 kg), compared to the control group (346 ± 11.4 kg) (Vodolazska and Lauridsen, 2020). This trend was not observed with the inclusion of 16 g/kg HS oil (Idricenau et al., 2021). Most probably, while a high lipid content in the diet of lactating sows allows an improvement in the growth performance of piglets, high milk production with a high lipid content affects the performance of sows, resulting in weight loss (Hansen, 2012). Another determining factor in weight loss is the oxidative stress faced by sows mainly related to the process of lipid peroxidation, which alters the antioxidant status of the animal. In particular, oxidative stress affects the ability of sows to produce milk, which, as previously reported, negatively limits the growth and performance of piglets. Persistent stress conditions lead to the formation of a large amount of free radicals and reactive oxygen species, which can affect the endogenous antioxidant system. In these situations, poor nutrient intake may be one of the main causes of systemic oxidative stress in sows (Palade et al., 2019).

In the study conducted by Palade et al. (2019), however, the inclusion of a dietary treatment with HSs (20 g/kg HS meal before farrowing; 50 g/kg during lactation) led to an improved antioxidant profile in the plasma of sows and piglets. According to the same authors, the positive correlation between HSs and antioxidant activity is due to the high content of PUFAs, which are known not only for their high nutritional profile but also for their ability to increase gene expression and the activity of several antioxidant/ detoxifying enzymes (Palade et al., 2019). This hypothesis was also

confirmed in the work of Vodolazska and Lauridsen (2020), in which the addition of HS oil (50 g/kg) resulted in elevated levels of eicosapentaenoic acid (C20:5, n-3) and Docosapentaenoic acid (C22:5, n-3), in the plasma of sows and piglets, as a result of the conversion of ALA and stearidonic acid to longer chain n-3 PUFAs. In fact, as reported by Farinon et al. (2020), these fatty acids are characterised not only by high inflammatory activity but also by an important antioxidant activity.

As reported earlier, there are few studies in the literature focusing on the use of hemp-based products in the pig sector, mainly considering the weaning period. As shown, inclusion levels of up to 50 g/kg of HSs in pregnant sows resulted in improved growth performance of treated piglets due to a better nutritional profile of the milk, richer in n-6 and n-3 PUFAs. Similar results were obtained with 50 g/kg of HS oil, although this inclusion led to a reduction in sow weight during pregnancy, a phenomenon not observed with the inclusion of 16 g/kg, suggesting that lower levels may benefit both lactating sows and piglet growth performance.

However, although all these results suggest that hemp-based products could be included in pig diets, further studies are needed to identify the optimal level of inclusion for each product to ensure the best animal health and performance.

Broilers

Interesting results were reported for broilers. More precisely, as observed in the study by Khan et al. (2010), the inclusion of 20 g/kg of HSs resulted in a better feed conversion ratio (**FCR**) with a higher slaughter weight compared to the control group (2087.2 \pm 10.25 and 1861.4 \pm 32.2 g/kg). As explained by the authors, this trend is due to the high lipid and AA profile of the HSs, confirming the results reported by Parr et al. (2020), where the inclusion of 20 g/kg proved to be better than 10 g/kg, 30 g/kg, and 40 g/kg to modulate the growth performance of broilers.

However, the inclusion of 40 g/kg, as reported by Skřivan et al. (2020), resulted in comparable results in terms of performance and product quality to flaxseeds, but added at 60 g/kg, confirming the high nutritional profile of HSs. This trend was also observed for bone health, a key parameter for animal welfare, especially under intensive livestock conditions. Most probably, as reported by Skřivan et al. (2020), this result is positively correlated with the presence of α -tocopherol, which is higher in both the diet and the meat of animals that received the HSs diet. Indeed, α -tocopherol supplementation tends to induce an increase in osteogenic bone mass in the vertebral secondary cancellous bone, where active bone remodelling occurs (Skřivan et al., 2020). However, although these results showed that an inclusion of up to 40 g/kg of HSs in broiler diets leads to positive results, it is necessary to consider how in the first weeks of the production cycle, the high fibre and mineral content could affect the health of the animals. Indeed, as reported by Vispute et al. (2019), at this stage, chickens have an underdeveloped mucosa, unable to produce sufficient enzymes capable of digesting high cellulose content.

The use of HS oil led to similar results with different concentrations. In particular, Jing et al. (2017) showed that the inclusion of 30 and 60 g/kg of HS oil in a control diet (based on corn oil) did not change the performance (BW, FI and FCR) of the treated animals, but increased the n-3 PUFAs content in the meat (71.4 \pm 14. 8 g/100 g and 148.9 \pm 14.8 g/100 g, respectively). The result was partially confirmed by Kanbur (2022). However, the author strongly advises against the use of HS oil, especially in the first three weeks of rearing, having recorded negative values for growth performance. In contrast to the reports of Vispute et al. (2019), where it was the high fibre content that affected growth performance, in this case, dealing with a lipid matrix is difficult to speculate on the causes. However, as reported by Fouad and ElSenousey (2014), a high inclusion of PUFAs in the diet of broilers can lead to fatty acid β -oxidation by reducing fat deposition.

The use of hemp co-products in the diets of monogastric animals is attracting increasing interest; however, to date, there is limited work in the literature. Stastnik et al. (2016) tested the use of 25 g/kg of HSs expellers and 10 g/kg hemp plant tops (flowers, HSs and a bit of shives) in the diet of broilers, observing no difference in carcass yield at slaughter, compared to the control diet (maize diet). However, the use of these hemp-based products has not yet been authorised by the European Union (EU, 2017), suggesting the need for further studies on the valorisation of these co-products. In parallel, the European Union approved the use of HSs cake, although conflicting results were observed for broilers. More specifically, Eriksson and Wall (2012) demonstrated how the inclusion of 200 g/kg of HSs cakes registered highly comparable values in growth performance parameters (FI, FCR, final weight) and mortality to diets based on soybean cakes, rapeseed cakes and peas. Although these results suggest the use of HSs cakes as an alternative to soybean cakes, Stastnik et al. (2015) reported opposite results with inclusions of 50 and 150 g/kg, causing a worsening in final weight compared to the control diet (soybean cake). Most probably, these effects can be attributed to the presence of anti-nutritional factors, especially non-starch polysaccharides, whose content at the fibrous level increases with the removal of the lipid fraction.

As shown in Table 5, the use of hemp-based products led to contrasting results. The inclusion of HSs recorded better values on growth performance (final BW) with an inclusion of 20 g/kg. Studies conducted on the inclusion of oil (up to 60 g/kg) in the diet of broilers revealed a highly improved PUFA profile in meat, although growth performance is often unaffected or depleted. The same applies to hemp co-products. Seed cake showed discordant results with the inclusion of 50, 150, and 200 g/kg on FCR and final BW, suggesting further investigation of these matrices to identify the best inclusion levels.

Laying hens

As shown in Table 6, the use of hemp-based products has been widely explored in the laying hens sector. The major studies, in fact, focus on the functional and nutritional characterisation of eggs as well as the evaluation of qualitative and quantitative parameters. In parallel, aspects such as production performance and animal welfare have been investigated.

Regarding the functional aspect of eggs, although Mierlită (2019) observed no differences in the total cholesterol content of egg yolk from hens treated with HSs (80 and 200 g/kg), studies by Shahid et al. (2015) and Skřivan et al. (2019) reported different trends. More specifically, Shahid et al. (2015) reported that increasing the inclusion of HSs (150, 200, and 250 g/kg) reduced the cholesterol content with significant differences $(16.91 \pm 0.01 \text{ mg/g}; 14.29 \pm 0.01 \text{ mg/g}; 14.29$ 01 mg/g; 11.65 \pm 0.01 mg/g) compared to the control (corn and soybean meal) (19.27 \pm 0.01 mg/g), respectively. Identical trends were observed by Skřivan et al. (2019), even with lower inclusions of HSs (30, 60, and 90 g/kg). This is in agreement with the work of Mahmoudi et al. (2015) and Vispute et al. (2019). Most probably, this positive effect is related to the presence of phytosterols, particularly β -sitosterol, which is able to reduce hypercholesterolaemia by blocking the absorption of cholesterol through coprecipitation and crystallisation. Furthermore, phytosterols with low water solubility compared to cholesterol can misplace cholesterol from intestinal micelles (Shahid et al., 2015).

Functionality also depends on the presence of antioxidant compounds. In this regard, it is important to point out that Skřivan et al. (2019) demonstrated that the inclusion of HSs resulted in an increase in tocopherol, an important antioxidant involved in

Effects of the inclusion of hemp-based products (HSs, HS oil, HSs cake, HSs expellers, hemp plant top) in the broiler diet. HSs: Hempseeds; FCR: Feed Conversion Ratio; FI: Feed Intake; PUFAs: polyunsaturated fatty acids.

Total number of animals	Experimental Period	Treatments	Effects	Ref	
160 broiler chicks	42 days	 Control group; 5, 10, 20 g/kg of HSs. 	Increased final BW gain (20 g/kg HSs); Increased FCR (20 g/kg HSs); Decreased FI (20 g/kg HSs); No differences in mortality;	Khan et al., 2010	
50 broiler chicks	49 days	Control group;10, 20, 30, 40 g/kg of HSs hearts.	Increased profit per chick. Increased final BW gain (20 g/kg HSs); Increased FCR (20 g/kg HSs).	Parr et al., 2020	
192 broiler chicks	42 days	 Control group; 2, 3 g/kg of HSs. 	Decreased BW gain and FI in the first 3 weeks; Decreased total cholesterol and triglyceride in serum.	Vispute et al., 2019	
540 cockerels	35 days	Control group;40 g/kg HSs.	No differences in BW; No differences in FI; No differences in FCR; No differences in PUFAs; Increased bone health.	Skřivan et al., 2020	
150 broiler chicks	21 days	Control group;30, 60 g/kg of HS oil).	No differences in BW; No differences in FI; No differences IN FCR; Increased n-3 PUFAs in thigh and breast meat.	Jing et al., 2017	
200 broiler chicks	21 days	 Control group; 61 g/kg HS oil. 	Decreased BW gain; Decreased FCR; Decreased FI; Increased FI; No differences in cholesterol and triglycerides.	Kanbur, 2022	
1200 chicks	70 days	 Control group; 100 g/kg (starter), 200 g/kg (grower) of HSs cake. 	No differences in FCR; No differences in FI; No differences in live BW; No differences in mortality rate.	Eriksson and Wall; 2012	
75 cockerels	35 days	 Control group; 150 g/kg, 50 g/kg of HSs cake. 	Decreased final BW; Decreased in FCR; No differences in % of breast and leg muscle.	Stastnik et al., 2015	
60 cockerels	27 days	 Control group; 25 g/kg of HSs expellers; 10 g/kg of hemp plant tops. 	No differences in final BW; No differences in carcass yield; No differences in microbial colonisation.	Stastnik et al., 2016	

the prevention of lipid peroxidation during the storage period. This result was also confirmed by Mierliță (2019). Indeed, as reported by the same author, eggs obtained from HSs-treated animals not only showed higher levels of α -tocopherol but also a lower level of malondialdehyde (a marker of lipid peroxidation) than the control group.

Another aspect to consider is the nutritional one. In general, eggs from animals treated with hemp-based products showed a better lipid profile. More precisely, hemp-based products tend to decrease the level of SFAs and MUFAs within the yolk in favour of PUFAs, as reported by Shahid et al. (2015). These results are mainly due to the high level of n-3 PUFAs following the treatments with $150 \text{ g/kg} (7.79 \pm 0.13 \text{ mg/g})$, $200 \text{ g/kg} (10.29 \pm 0.09 \text{ mg/g})$, and $250 \text{ g/kg} (15.11 \pm 0.81 \text{ mg/g}) \text{ of HSs compared to the control} (2.6)$ 6 ± 0.17 mg/g), resulting in an improved n-6/n-3 ratio (shahid et al., 2015). Similar results were observed both by Jing et al. (2017) with the inclusion of HSs in hemp diets of 40 and 80 g/kg and by Neijat et al. (2016). The latter observed that the inclusion of up to 300 g/kg of HSs and up to 90 g/kg of HS oil, although not yielding statistically significant differences in total SFAs content, did decrease MUFAs content by increasing PUFA content, particularly n-3 PUFAs, improving the n-6/n-3 ratio. Specifically as reported by Gakhar et al. (2012), the main n-3 fatty acid subject to an increase is ALA. The authors showed how treatments with 100 and 200 g/kg HSs resulted in higher levels of this fatty acid of 51.7 \pm 3.23 and 91.3 \pm 3.23 mg/g, respectively, compared to the control 15.8 ± 3.23 mg/g. Results were also confirmed in diets with the inclusion of 40, 80 and 120 g/kg HS oil. The high ALA content plays a key role, as ALA can be converted to docosahexaenoic acid by the metabolism of chickens, the positive role of which has been explained above. Specifically, docosahexaenoic acid is derived from ALA, following the sequential reaction of the enzymes desaturase and elongase and β -oxidation, with eicosapentaenoic acid and Docosapentaenoic acid as intermediates. These reactions explain why high levels of docosapentaenoic acid, eicosapentaenoic acid and docosahexaenoic acid are found in the eggs of these animals, despite the fact that HSs lack them. In fact, as shown by Gakhar et al. (2012), treatments with HSs (100 and 200 g/kg) resulted in an increase in eicosapentaenoic acid $(0.9 \pm 0.08 \text{ mg/g})$ 1.2 ± 0.08 mg/g, respectively) compared to control (diet based on soybean meal and corn oil) $(0.2 \pm 0.08 \text{ mg/g})$ and docosahexaenoic acid (39.2 ± 1.64 mg/g, 47.4 ± 1.64 mg/g) compared to control $(17.1 \pm 1.64 \text{ mg/g})$. These results are also confirmed in studies conducted by Goldberg et al. (2012) and Rajasekhar et al., (2021b). Interesting observations were made by Konca et al. (2019).

Other aspects to consider are the physical parameters of eggs, such as colour, weight, shell thickness and breakage. Although colour is only a minor aspect, it is of fundamental importance in the consumer's perception. However, it is complicated to identify colour as a quality parameter, as it depends on individual preferences (Konca et al., 2019). As shown in Table 6, the addition of hempbased products resulted in changes in egg yolk colour (Skřivan et al., 2019; Konca et al., 2019; Goldberg et al., 2012; Park et al., 2014). As reported by Goldberg et al. (2012), the intensity of yolk colour is mainly due to the presence of yellow and red oxy-carotenoids, or xanthophyllic pigments, in the diet of hems.

Effects of the inclusion of hemp-based products (HSs, HS oil, HSs cake) in the laying hens' diet. HSs: Hempseeds; SFAs: saturated fatty acids; PUFAs: polyunsaturated fatty acids; MUFAs: monounsaturated fatty acids.

Total number of animals	Experimental Period	Treatments	Effects	Ref
60 hens	21 days	 Control group; 	Decreased cholesterol level in egg yolk;	Shahid et al., 2015
		• 150, 200, 250 g/kg of	Decreased SFAs in egg yolk;	
		HSs.	Increased PUFAs in egg yolk;	
			Increased n-3 in egg yolk.	
240 hens	84 days	 Control group; 	Decreased cholesterol level in yolk egg;	Skřivan et al., 2019
		• 30, 60, 90 g/kg of HSs.	Increased tocopherol level in egg yolk;	
		,, 8,-8	Increased yellowness with an inclusion of 30 and 60 g/kg of HSs respectively;	
			Increased laying rate for 30 g/kg HSs;	
			Decreased shell thickness;	
			Increased tibia strenght.	
108 hens	84 days	 Control group; 	Increased PUFAs in egg yolk;	Konca et al., 2019
		 150 g/kg of raw HSs; 	Decreased SFAs (only for heat-treated HSs) in egg yolk;	
		• 150 g/kg heat-treated	Decreased MUFAs in egg yolk;	
		HSs.	Increased egg yolk colour in raw HSs;	
			No differences in egg weight;	
			No differences in laying rate.	
40 hens	42 days	 Control group; 	No differences in MUFAs in egg volk;	Jing et al., 2017
40 110113	42 duy3	• 40, 80 g/kg of HS oil.	No differences in SFAS in egg yolk;	Jing et al., 2017
			Increased levels on n-3 in egg yolk;	
			No differences in laying rate.	
40 h an a	0.4 days	Control mount		Nellet et al. 2010
48 hens	84 days	Control group;	No differences in SFAs in egg yolk;	Neijat et al., 2016
		• 100, 200, 300 g/kg of	Decreased MUFAs in egg yolk;	
		HSs;	Increased n-3 PUFAs in egg yolk.	
		• 45, 90 g/kg of HS oil.		
48 hens	84 days	Control group;	No differences in egg weight;	Neijat et al., 2014
		• 100, 200, 300 g/kg of	No differences in eggshell thickness;	
		HSs;	No differences in laying rate.	
		 45, 90 g/kg of HS oil. 		
48 hens	84 days	 Control group; 	Increased n-3 PUFAs in egg yolk;	Gakhar et al., 2012
		 40, 80, 120 g/kg HS oil; 	Increased egg weight only for 200 g/kg of HSs;	
		 100, 200 g/kg HSs). 	No differences in egg mass production;	
			No differences laying rate.	
48 hens	84 days	 Control group; 	Increased n-3 PUFAs;	Goldberg et al., 2012
		• 40, 80, 120 g/kg of HS	Increased n-6 PUFAs;	
		oil;	Increase egg yolk redness and yellowness.	
		 100, 200 g/kg of HSs. 		
30 hens	35 days	Control group;	Decreased total cholesterol level and triglycerides in serum;	Park et al., 2014
	0	• 16.8 g/kg of HS oil.	Increased egg yolk colour;	
		0, 0,	Increased laying rate.	
102 hens	28 days	 Control group; 	No differences in egg weight;	Silversides and
	20 days	• 50, 100, 200 g/kg of HSs	No differences in laying rate.	Lefrançois, 2005
		cake.	no uncrences in laying fuce.	Lenunçois, 2005
216 hens	169 days		No differences in any weight:	Halle and Schöne, 2013
	168 days	 Control group; 50, 100, 150 g/kg of HSs 	No differences in egg weight; Increased egg mass production.	riane and Schone, 2013
		• 50, 100, 150 g/kg 01 H5s cake.	nicieaseu egg mass production.	
800 hens	112 days	 Control group; 	No differences in laying rate.	Rajasekhar et al.,
800 Hells	112 uays	e	No unerences in laying fate.	
		• 100, 200, 300 g/kg of HSs		2021a
0001	110.1	cake.		B 1 11 1 1
800 hens	112 days	Control group;	Decreased MUFAs in egg yolk;	Rajasekhar et al.,
		 100, 200, 300 g/kg of HSs 	Increased PUFAs;	2021b
		cake.	No detection of cannabinoids.	
120 hens	70 days	 Control group; 	No differences in cholesterol the level in egg yolk;	Mierliță, 2019
		 80 g/kg of HSs; 	Increased tocopherol lever in egg yolk for HSs;	
		 200 g/kg of HSs cake. 	Increased egg weight with the inclusion of HSs;	
			No differences in laying rate.	

Depending on the level of these compounds in the diet, the colour of the yolk can vary from almost absent to an intense orange. However, it is important to emphasise that where changes in yolk colour are statistically significant, the perception to the human eye may be relatively insignificant and these results cannot infer consumer preferences.

Egg weight, on the other hand, represents a quality parameter. Most likely, the increase in egg weight is directly related to the increase in yolk weight, at the expense of other egg components. As shown in Table 6, the addition of hemp-based products not only resulted in comparable results to the control eggs (Halle and Schöne, 2013; Silversides and Lefrançois, 2005; Neijat et al., 2014) but also in better results. More specifically, in the study conducted by Mierliță (2019), the supplementation of 80 g/kg HSs resulted in higher weights (61.7 ± 0.937 g) than eggs from animals treated with 200 g/kg HSs cake (58.3 ± 0.937 g) and control animals. Similar results were also observed by Gakhar et al. (2012). Indeed, animals treated with an inclusion of up to 200 g/kg HS-produced eggs characterised by a weight of 60.57 ± 1.14 g, higher than those treated control (56.2 ± 1.14 g).

In parallel, eggshell thickness is another key parameter. Plasma Ca and P in laying hens are interrelated. The Ca and P content in the diet has an effect on the body's effort to regulate blood pH with a consequence on the acid-base balance (Keshavarz, 1994). In laying

hens, maintaining an optimal acid-base balance is necessary for bicarbonate homeostasis and eggshell formation. Differences in eggshell quality have been attributed to changes in the mineral profile of the hens' blood plasma (Keshavarz, 1994). However, as reported by Neijat et al. (2014), it is difficult to find a direct link between dietary treatment and the content of these minerals in the blood. Most likely, as the authors argue, their levels reflect changes in demand with age. For example, Ca requirements are highest during peak egg production and may be reflected by serum/plasma calcium levels. The increase over time, irrespective of diet, may reflect a relative excess of Ca and P intake over metabolic requirements as the hens exceed peak production. However, as shown in Table 6, treatment with HSs had discordant results. While Park et al. (2014), Neijat et al. (2014) observed no difference compared to the control groups, Skřivan et al. (2019) showed a negative correlation as HS inclusion increased. In particular, the control group showed an eggshell thickness of $363 \pm 1.5 \mu m$, which was comparable to the diets containing 30 g/kg (357 \pm 1.5 μ m) and $60 \text{ g/kg} (357 \pm 1.5 \mu \text{m}) \text{ of HSs, but higher than the diet with } 90 \text{ g/kg}$ of HSs (350 ± 1.5 µm).

These minerals also play a key role in bone health. As observed by Skřivan et al. (2019), the higher tibial strength is in agreement with the higher Ca level in groups fed HSs-enriched diets, up to 90 g/kg. At the same time, Cannabidiol and α -tocopherol also produce positive results. The former improves fracture healing by acting on collagen cross-linking, while the latter, as previously reported, tends to induce an increase in osteogenic bone mass in vertebral secondary cancellous bone, where active bone remodelling takes place (Skřivan et al., 2019).

As far as the production parameters of laying hens are concerned, the laying rate is certainly the most important. As shown in Table 6, the results obtained are very interesting, but require further investigation. In general, the inclusion of hemp-based products has enabled production rates to be matched or improved, with no negative trends. If in the studies conducted by Jing et al. (2017), Neijat et al. (2014), Gakhar et al. (2012), Mierliță (2019), Konca et al. (2019), and Rajasekhar et al., (2021a), no differences were observed compared to the control groups, interesting results were recorded by Skřivan et al. (2019), Park et al. (2014) and Halle and Schöne (2013). More precisely as reported by Skřivan et al. (2019), the laying rate was improved following the 30 g/kg HSs treatment (93.6 \pm 0.59%) compared to the control group and the 60 and 90 g/kg treatments (88.7 \pm 0.59%, 86.4 \pm 0.59%, 89.49 \pm 0. 59%, respectively). These results were also confirmed by Halle and Shone (2013) with a supplementation of 100 and 150 g/kg HSs cake compared to flaxseeds cake. Park et al. (2014) showed that 16.8 g/kg HS oil increased the deposition rate (97.86 ± 0.250 %) compared to the control group (96.07 ± 0.250%). Although it is difficult to hypothesise the explanation for this effect, it is very likely that hemp-based products have nutrigenomic value by modulating the genes responsible for oviposition rates.

As just reported, the use of hemp-based products has resulted in largely positive results in laying hens, both in the functional and nutritional aspects of eggs, animal health and growth performance. These positive effects were recorded with different levels of HSs inclusion (up to 250 g/kg). In parallel, the use of HS oil increased the nutritional profile of eggs by raising the PUFAs present, with an inclusion of up to 300 g/kg. Interesting results were also reported for hemp co-products, in particular HSs cake (up to 150 g/kg) improving laying hen production. These findings add to the knowledge reported by EFSA (2011), in which the use of HSs and HSs cake were confirmed up to 20% and up to 12% for HS oil, without altering the performance of the animals and the sensory characteristics of the eggs.

Conclusion

The inclusion of hemp-based products in the diets of monogastric animals has led to different results depending on the species considered. As reported, the use of these products needs further investigation, especially in pigs and broilers, due to limited studies. However, for these species, this review increased the data shown in the last EFSA report by reporting for the first time considerations for the use of new matrices such as HS oil. For laying hens, on the other hand, the large number of scientific studies has made it possible to identify and confirm hemp-based products as effective and safe matrices, able to positively modulating animal health and performance, while simultaneously enhancing the nutritional and functional profile of eggs. However, even in this case, the choice of the optimal inclusion of hemp-based products requires further evaluation.

Ethics approval

Not applicable.

Data and model availability statement

None of the data were deposited in an official repository. Information can be made available from the authors upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

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Declaration of interest

None.

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