Hemp for Dairy Ruminants

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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. 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.

hemp dairy ruminants milk yield milk composition fatty acid profile

1. 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].

2. Use of Hempseed and Derivatives in Dairy Ruminants

2.1. 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][49][50][51][52][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 1). 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 1. 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

Groups	HC Dosage (% DM)	Milk Yield (kg/d)	Milk Protein (%)	Milk Fat (%)	Milk Urea (mmol/l)	Protein Efficiency [1]
HC32	31.8	26.8	3.40	3.89	5.1	0.22
p-value [2]	[54]	0.022	0.028	n.s.	<0.001	0.009

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

ws of four
emp 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 carinata 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.

2.2. Use of 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 2). 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 3). The alpha-tocopherol and antioxidant activity increased using hempseed in the diets, reducing the risk of lipid oxidation in raw milk.

Table 2. 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

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.

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 2). 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 3). As known, the availability of precursors (i.e., linoleic acid) for ruminal bio-hydrogenation and synthesis of CLA is high at pasture when the animals were fed fresh forage [61].

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 2). 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.

2.3. Use of 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 4).

Table 4. 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	

inseed or seed and d in milk protein, lactose and urea concentration (Table 4).

[1] n.s. = not significant.

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 5). 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 5), 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 5. 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.

2.4. Use of 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.