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Article

Diet Supplementation with Hemp (*Cannabis sativa* L.) Inflorescences: Effects on Quanti-Qualitative Milk Yield and Fatty Acid Profile in Grazing Goats

Ruggero Amato [1], Marianna Oteri [2], Biagina Chiofalo [2], Fabio Zicarelli [1], Nadia Musco [1], Fiorella Sarubbi [3], Severina Pacifico [4], Marialuisa Formato [4], Pietro Lombardi [1], Federica Di Bennardo [1], Piera Iommelli [1,*], Federico Infascelli [1] and Raffaella Tudisco [1]

[1] Department of Veterinary Medicine and Animal Production, University of Napoli Federico II, 80100, Napoli, Italy; ru.amato@outlook.com; nadia.musco@unina.it; pilombar@unina.it; dibennardofederica@gmail.com; piera.iommelli@unina.it; infascel@unina.it, tudisco@unina.it

[2] Department of Veterinary Sciences, University of Messina, 98122 Messina, Italy; marianna.oteri@unime.it; biagina.chiofalo@unime.it

[3] Institute for the Animal Production System in the Mediterranean Environment, National Research Council, 80055 Portici, Italy; fiorella.sarubbi@cnr.it;

[4] Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania Luigi Vanvitelli, Via Vivaldi 43, 81100 Caserta, Italy; severina.pacifico@unicampania.it; marialuisa.formato@unicampania.it

* Correspondence: Corresponding author: piera.iommelli@unina.it

Simple Summary: The aim of the present work was to evaluate the effect of hemp inflorescences supplementation in grazing goats' on milk yield and nutritional characteristics. Hemp (*Cannabis sativa L.*) is a plant which showed several beneficial properties in human and animal when used as supplement thanks to its nutritional characteristics. In our trial 20 goats were recruited and divided into two groups: group control was mainly fed grazing pasture (G) and group treated (GH) received in addition 20g/head/day of *Cannabis sativa* inflorescences; both groups received 700 g/head/day of a mixed concentrate. Individual milk samples were collected every 20 days and were analysed for chemical composition and fatty acid profile. Results showed no differences in milk yield and chemical composition of the two groups while several differences in fatty acid profile were observed. Lauric acid (C12:0) was significantly higher in milk of group GH (4.83% vs 4.32%; P<0.01) as well as total conjugated linoleic acids (CLAs) (0.435% vs 0.417%; P<0.01).

Abstract: Hemp (*Cannabis sativa L.*) is an annual plant belonging to the family of Cannabaceae with several varieties characterised by different fatty acid profiles, content in flavonoids, polyphenols and cannabinoid compounds. Hemp is mostly used in livestock nutrition as oil or as protein cake, not as inflorescences. The aim of this study was to evaluate the effect of dietary hemp inflorescences on milk yield and composition in grazing goats. Twenty goats (Camosciata delle Alpi), after kidding, were equally divided into two groups (G: Grazing and GH: grazing and hemp), homogeneous for milk yield in the previous lactation, parity and live weight. For three months, all goats were fed on a permanent pasture and received 700/head/day of concentrate (barley, oats and faba bean); diet of group GH was supplemented with 20 g/head/day of hemp inflorescences. Pasture DM intake was estimated according to previous research performed in the same area. In addition, goats' body weight did not change along the trial meaning that their energy requirements were guaranteed. Individual milk yield was daily registered and samples collected every 20 days for chemical composition (Milkoscan) and fatty acid profile (Gas Chromatography). The data were analysed by ANOVA (GLM procedure of SAS, 2000), for repeated measure. No significant differences were found for milk yield and chemical composition. Lauric acid (C12:0) was significantly higher in milk of group GH (4.83% vs 4.32%; P<0.01) as well as total conjugated linoleic acids (CLAs) (0.435% vs 0.417%; P<0.01).

Keywords: hemp; dairy goats; fatty acid profile

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1. Introduction

Cannabis sativa L. is an annual plant of the family Cannabaceae which grows roughly anywhere and it includes various botanical subspecies [1]. *Cannabis sativa var. sativa* has gained particular interest in the last years for its low content in tetrahydrocannabinol (THCA) (<0.2–0.3%), a chemical compound responsible for some psychoactive effects, which makes this cultivar suitable for legal cultivation in Europe [2]. For its nutritional characteristics this plant has been studied as feasible feed supplement in various animal species [3–5]. As a matter of fact, hemp is characterized by a great content in polyunsaturated fatty acids (PUFA) reaching almost the 80% of total fatty acids (FA), manly linoleic (LA) and alpha-linolenic acids (ALA) [6–8]. Feeding animals with sources of PUFA enhances the concentration of beneficial FA in animal products with positive effects on human health [9]. In this regard, LA e ALA represent the main susbstrate for rumen biohydrogenation [10] which leads to the production of intermediated by-products in milk such as conjugated linoleic acids (CLAs) known for their anticarcinogenic properties [11]. Additionally, hemp is also rich in bioactive compounds such as natural antioxidants (polyphenols, tocopherols, carotenoids and phytosterols) [12] which, according to Poulopoulou et al. [13], positively influence milk fatty acids profile, increasing the production of total fatty acids, specifically the concentration of the beneficial fatty acids.

The use of plant-based feed supplements has been seen to improve the quality of goat milk and their derived products [14,15] because of the natural polyphenols and antioxidant compounds having a potential therapeutic benefit such as anticarcinogenic and antiviral properties, which have been observed in both in vitro and human assays [16]. Moreover, the nutritional characteristics of animal products are important aspects for the consumers, namely factors that influence consumers at the time of purchase [17]. The most used parts of the hemp in livestock nutrition are the seeds [8] and the protein cake resulting as by-products by squeezing the seeds for obtaining oil [18] and as far as we know, no works have been done on the use of the inflorescences in animal nutrition.

Thus, this paper aimed to investigate the effects of feeding grazing goats with hemp inflorescences, on milk yield and quality.

For the purpose *Cannabis sativa* variety Kompolti has been used in the trial. Kompolti variety origins in Hungary, is mainly cultivated for inflorescences and because of its resistance to low temperature can support open cultivation, while most of hemp variety cannot.

2. Materials and Methods

2.1. Animals and diet

The trial was performed according to the Animal Welfare and Good Clinical Practice (Directive 2010/63 /EU) and was approved by the local Animal Ethic Committee (protocol number: PG/2019/0070006) at the "Funky Farm", located in Sant'Apollinare (FR, Italy) at 400 meters a.s.l. (41 ° 14 'N; 13 ° 50'E), with an average rainfall of 530 mm and an average annual temperature of 6 - 23 ° C.

Twenty pluriparous goats (Camosciata delle Alpi; body weight (BW) 45 kg (± 2.0 kg)), immediately after kidding (first week of March) were equally divided into two groups (G: grazing; GH: grazing and hemp) homogeneous for parity (3rd) and milk yield in the previous lactation (g/head/day 2000 ± 120). Both groups had free access to water and to a permanent pasture (8:00 am to 3:00 pm and 5:00 pm to 20:00 pm), mainly composed by the spontaneous vegetation of the area (*Trifolium alexandrinum, Vicia spp:, Crataegus monogyna, Rubus ulmifolius, Clematis vitalba, Medicago sativa, Festuca arundinacea, Bromus catharticus, Festuca arundinacea* and *Lolium perenne*) and, when in the pen, received 700 g/head/day of concentrate composed by barley (23%), oats (22%) and faba beans (55%); group GH received in addition 20 g/head/day of Cannabis sativa Kompolti inflorescences.

The hemp used in this trial was produced by the "Centro Sviluppo Canapa del Sud" in Campania, Italy. Inflorescences were gathered at a flowering stage and then dried at 21–23° C for 10 days; subsequently, the flowers were trimmed.

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2.2. Feed sampling and analysis

In order to reduce the differences in chemical composition observed in studies based on a monthly collection [19,20], in this trial pasture samples were collected weekly. Every week (from May to July) pasture was sampled from four different areas measuring 2.5 m2, cutting at 3 cm from the ground and then, after oven dried at 65°C and weighing, the 4 weekly representative samples (1 kg each obtained balancing the amount from the 4 different areas) were pooled for a monthly analysis. Hemp and concentrate were sampled in triplicate only at the beginning of the trial. Pasture samples were milled through a 1 mm screen and, as well as concentrate and hemp samples, analysed for chemical composition according to AOAC [21] whereas the structural carbohydrates were determined as suggested by Van Soest et al. [22] and nutritive value (UFL=1700 kcal of net energy for lactation) was calculated according to INRAE [23]. Feed fatty acid (FA) profile was determined by gas chromatography (GC).

Around 200 mg of fat, extracted according to Folch et al. [24], were directly methylated as suggested by Christie [25]. In brief, the lipid extract was added with 2 mL of a methanol:sulfuric acid (9:1, v/v) mixture, heated in air-oven (100 °C for 1 h), cooled at room temperature and mixed with 1 mL of n-hexane. Successively, the solution was filtered (0.45 µm filter) and injected into a GC with flame ionization detector (FID). The analyses of fatty acid methyl ester (FAME) were performed by a GC-FID (TRACE 1310) system equipped with an AI 1310 Auto-injector/AS 1310 Autosampler (Thermo Fisher Scientific, Milan, Italy). A capillary polar column Omegawax 250 (Supelco, Bellefonte, PA, U.S.A.), 30 m × 0.25 mm, 0.25 µm (L × I.D., film thickness) was adopted with the following oven temperature program: 100 °C for 5 min, 100 - 240 °C at 4 °C/min, final isotherm at 240 °C for 20 min. Injector and detector temperature, 250 °C; injection volume, 0.5 µL; split ratio, 1:50. Carrier gas (He) at a flow rate of 1 mL/min; make-up gas (N2) flow, 40 mL/min; H2 flow, 35 mL/min; air flow, 350 mL/min. Data were processed with the ChromeleonTM Data System (Thermo Fisher Scientific, Milan, Italy) software (Version 7.2.9) and individual FAMEs identified by comparing sample peak retention times with standards from Supelco (Merck KGaA, Darmstadt, Germany). Single FA concentrations were expressed as g/100 g, considering 100 g the total of all areas of the identified FAMEs.

2.3. Milk sampling and analysis

Starting from sixty days after kidding (May), individual milk yield was daily registered and individual milk samples, obtained by weighting the morning and the evening milkings, were collected every 20 days until July (total of 5 sampling). Milk chemical composition was evaluated using Milko Scan 133B (Foss Matic, Hillerod, Denmark) standardized for goat milk.

Milk FA profile was determined by extraction of total fat with a hexanopropanol and isopropanol (3/2 v/v) [26] mixture and subsequent trans methylation [27] modified by Chouinard et al. [28].

The methyl esters were quantified by GC (ThermoQuest 8000TOP gas chromatograph, Thermo Electron Corporation, Rodano, Milan) with flame ionization detector and with capillary column (CP-SIL 88 fused silica capillary column, 100 m x 0.25 mm internal diameter with 0.2-µm film thickness; Varian, Inc. Walnut Creek, CA) adopting the following temperature ramp:

70 ° C for 4 min → 13 ° C / min → 175 ° C for 27 min → 3 ° C / min → 215 ° C for 38 min → 10 ° C / min → 70 ° C.

The temperature of injector and detector were at 250 ° C and 260 ° C, respectively. Gas flows were: carrier gas (helium) 1 ml/min; hydrogen 30 ml/min; air 350 ml/min; make-up gas (helium) 45 ml/min.

FA peaks were identified by comparing with a standard mixture of fatty acid methyl esters (Larodan Fine Chemicals, AB, Limhamnsgårdens Malmö, Sweden). CLA isomers were identified by comparing samples chromatograms with those of single purified isomers (CLA cis-9, trans-11; CLA trans-10, cis-12; CLA cis-9, trans-11; CLA trans -9, trans-11) (Larodan Fine Chemicals, AB, Limhamnsgårdens Malmö, Sweden).

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2.4. Statistical analysis

Feed data were analysed using the one-way ANOVA with JMP software (version 11, PROC GLM, SAS 2000) according to the following model:

$$Yij = \mu + Si + \varepsilon j$$

where Yij = mean of response variable, μ = general mean, Si = sampling effect (i = 3; May, June, July), and εj = experimental error.

Body weight and milk data were analysed using the two-way ANOVA for repeated measures with JMP software (version 11, PROC GLM, SAS 2000) according to the following model:

$$Yijk = \mu + Di + Sj + (DS)ij + \varepsilon ijk$$

where y = mean of response variable, μ = general mean, Di = effect of the dietary treatment (i = 2; G and GH), Sj = sampling effect (j milk = 5; I, II, III, IV, V), DS = interaction between dietary treatment × sampling effect, and εijk = residual error. The means were statistically compared using Tukey's test. Differences were considered statistically significant at $p < 0.05$.

3. Results

Feeds chemical composition is reported in Table 1. Hemp was characterized by a high crude protein and ash content. The latter result could be explained by the harvesting technique of hemp which let soil be collected in the cutting phase. These parameters were in accordance with Vastolo et al. [4].

Table 1. Chemical composition (g/kg DM; mean ± SD) and energy value of feed.

	Pasture	Concentrate	Hemp
Crude Protein	159.0 ± 1.2	186.0 ± 1.7	261.3 ± 1.0
Ether extract	23.0 ± 0.3	34.2 ± 1.8	28.0 ± 0.2
NDF	487.1 ± 5.6	267.1 ± 3.3	405.9 ± 3.2
ADF	351.0 ± 2.7	108.1 ± 1.1	310.0 ± 1.9
ADL	53.2 ± 1.7	36.3 ± 0.9	163.3 ± 2.4
Ash	69.8 ± 1.4	11.2 ± 1.3	162.8 ± 2.4
UFL/kg DM	0.78	1.1	0.78

NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; UFL: Unit Feed for Lactation

Feeds FA profiles are reported in Table 2. Both pasture and hemp show a high value in PUFA especially in omega-6 and also omega-3. As we expected by our previous studies on Mediterranean pasture [29,30] and by literature on hemp [31], they show an opposite ratio between n6/n3, anyway guaranteeing both a significant amount of precursors for rumen biohydrogenation.

Table 2. Fatty acid profile of hemp and pasture along the trial (% of total FA).

	Concentrate	Hemp	Pasture		
			May	June	July
SFA	22.9 ± 0.8	10.1 ± 0.2	18.4 ± 0.2	17.2 ± 0.8	19.6 ± 0.5
MUFA	25.4± 1.1	17.03 ± 0.5	4.05 ± 0.05	4.03 ± 0.08	4.1 ± 0.03
PUFA	51.7± 3.3	70.09 ± 1.3	76.8 ± 1.02 ^{B}	79.4 ± 0.9^{A}	75.7 ± 0.78 ^{B}
LA	45.8± 2.8	43.9 ± 0.07	23.1 ± 0.07 ^{B}	33.7 ± 0.5 ^{A}	20.4 ± 0.2 ^{B}

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ALA	2.8 ± 0.2	26.3 ± 0.02	41.4 ± 0.08 ^{B}	42.8 ± 0.2 ^{A}	41.1 ± 0.2 ^{B}

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; LA: linoleic acid; ALA: alpha-linolenic acid. A and B in superscript within the rows differ significantly (p<0.001)

Goats BW did not change between the groups along the trial. Milk yield was not significantly different between the groups (g/head/day 2436.25 vs 2076.75, for the group T and C, respectively). Similarly, no differences were found on milk chemical composition (Table 3).

Table 3. Milk yield (g/d) and chemical composition (%).

	Treated group	**Control group**	**RMSE**
Body weight (kg)	45.3	45.2	5.10
Milk Yield	2336	2276	663.8
Protein	3.39	3.39	0.179
Fat	3.68	3.63	0.438
Lactose	4.91	4.86	0.252

RMSE, root mean square error.

In Table 4 the mean values of milk FA are reported. Among SFA, caproic (C6:0) and lauric acid (C12:0) show significantly higher value (p < 0,001) in group fed with hemp, whereas among the unsaturated fatty acids, trans vaccenic acid (C18:1 trans 11) and alpha linolenic (C18:3) acid were significantly lower (p < 0,001) in group GH, while adrenic acid (AdA; C22:4) resulted significantly higher (p < 0,001).

Table 4. Milk fatty acids (% of total FA).

	Treated	**Control**	**Group effect**	**Time effect**	**Interaction**	**RMSE**
C4:0	1.434	1.566	0.038	<0.0001	0.01	0.308
C6:0	1.809	1.742	<0.0001	<0.0001	<0.0001	0.245
C8:0	2.967	2.935	0.598	<0.0001	<0.0001	0.303
C10:0	11.77	11.54	0.262	<0.0001	<0.0001	0.968
C11:0	0.195	0.177	0.08	0.416	0.0028	0.049
C12:0	4.828	4.391	0.0003	0.0134	0.0007	0.575
C13:0	0.056	0.047	0.0365	0.0223	0.0013	0.021
C14:0	10.33	10.14	0.077	0.003	0.287	0.517
C14:1	0.077	0.079	741	<0.0001	0.258	0.031
C15:0	0.451	0.447	0.846	0.348	0.136	0.093
C16:0	26.85	27.74	0.0149	<0.0001	0.958	1.754
C16:1	0.223	0.247	0.033	<0.0001	0.014	0.054
C17:0	0.339	0.372	0.019	0.01	0.053	0.066
C17:1	0.029	0.027	0.407	<0.0001	0.64	0.014
C18:1 CIS6	0.043	0.030	0.04	0.069	0.129	0.03
C18:0	14.82	14.88	0.938	0.0008	0.042	1.465
C18:1 trans 9	0.258	0.299	0.012	<0.0001	0.778	0.078
C18:1 trans 11 (TVA)	1.69	2.06	<0.0001	0.0041	0.025	0.414
C18:1 CIS9	16.47	16.11	0.257	0.0082	<0.0001	1.538
C18:1 CIS10	0.334	0.348	0.501	<0.0001	0.027	0.093
C18:1 CIS11	0.308	0.359	0.0016	<0.0001	0.207	0.076
C18:1 CIS12	0.125	0.138	0.078	<0.0001	0.014	0.034
C18:2 trans N6	0.126	0.138	0.212	<0.0001	0.044	0.046

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C18:2 CIS N6 (LA)	2.04	1.93	0.047	<0.0001	0.262	0.267
C20:0	0.167	0.165	0.736	0.0243	0.026	0.024
C18:3 N6	0.032	0.040	<0.0001	0.0116	0.0006	0.009
C20:1	0.036	0.036	0.826	0.0003	0.011	0.01
C18:3 N3 (ALA)	0.754	0.792	0.166	<0.0001	0.738	0.133
C18:2 cis 9 trans 11	0.286	0.279	0.671	<0.0001	0.047	0.085
C18:2 trans 10 cis 12	0.148	0.141	0.28	<0.0001	0.005	0.033
C20:2 N6	0.028	0.027	0.733	0.0054	0.0028	0.0084
C22:0	0.067	0.064	0.405	<0.0001	0.101	0.0189
C22:1	0.013	0.017	0.014	<0.0001	0.023	0.0076
C20:3 N3	0.011	0.01	0.664	<0.0001	0.386	0.0058
C20:4 N6 (AA)	0.096	0.105	0.075	<0.0001	<0.0001	0.024
C22:2 N6	0.017	0.015	0.448	0.023	0.308	0.0123
C24:0	0.016	0.016	0.709	<0.0001	0.844	0.0039
C20:5 N3 (EPA)	0.048	0.05	0.63	<0.0001	0.002	0.0135
C24:1	0.02	0.021	0.829	0.249	0.041	0.0083
C22:4 N6	0.046	0.031	<0.0001	0.0001	0.763	0.0143

RMSE. root mean square.

The FA classes and the nutritional indices are reported in Table 5. The total CLA content was higher in group fed with hemp ($p < 0,001$), as well as the omega-6/omega-3 and the LA/ALA ratio ($p < 0,05$ and $p < 0,01$ respectively).

Table 5. Milk fatty acid classes and ratios.

	Treated	Control	Group effect	Time effect	Interaction	RMSE
SFA	76.127	76.278	0.6996	0.068	0.0004	1.9078
MUFA	19.627	19.722	0.786	0.0221	<0.0001	1.718
PUFA	3.643	3.575	0.436	0.288	0.97	0.42
omega-6	2.395	2.304	0.122	<0.0001	0.471	0.286
omega-3	0.812	0.854	0.121	<0.0001	0.673	0.132
ΣCLA	0.435	0.417	<0.0001	<0.0001	0.026	0.11
PUFA/SFA	0.047	0.047	0.523	0.406	0.777	0.0064
omega-6/omega-3	3.17	2.93	0.016	<0.0001	0.113	0.493
LA/ALA	2.96	2.68	0.005	<0.0001	0.091	0.477
AA/EPA	2.15	2.32	0.318	0.007	0.805	0.839

RMSE. root mean square; SFA. Saturated fatty acids; MUFA. Monounsaturated fatty acid; PUFA. Polyunsaturated fatty acids; CLA. Conjugated linoleic acid; LA. Linoneic acid; ALA. alpha-linolenic acid; AA. Arachidonic acid; EPA. Eicosapentanoic acid.

4. Discussion

Body weight didn't change along the trial in both the experimental groups. The diet of all the grazing goats were formulated to satisfy their energy requirements according to Tudisco et al. [32] as follows: energy requirements for maintenance 0.0365 UFL/kg metabolic weight (MW:BW0.75); energy requirements for milk production 0.41 UFL/kg fat-corrected milk (FCM, 4% fat). Therefore, total energy requirement was 1.48 UFL (0.63 UFL maintenance + 0.85 UFL milk production) and since the average pasture DM intake in the inlands of South Italy is 20g/kg BW [33] the deficit of 0.78 UFL was met by the concentrate.

Pasture 's nutritional characteristics, both chemical composition and fatty acids profile are similar to those reported in previous studies on spontaneous pasture of Mediterranean areas [34,35]. The most abundant fatty acids in all pasture samples was C18:3 and on the contrary of what observed

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by Meľuchová et al. [36], it remains high in all sampling months. The study of Meľuchová et al. [36] showed a decrease in C18:3 content from May to July and an increase in C18:2 that we only detected in June. As the authors suggested, these great differences in term of C18:3 and C18:2 are mainly derived by the different plants composing the pasture in the sampling months and their different phenological phase. As a matter of fact, the phenological phases can influence the fatty acid composition of some plants as showed by Cabiddu et al. [34] who analysed FA composition of different botanical species composing the pasture, in different phenological stages (reproductive and growth).

With regards to hemp inflorescences' nutritional characteristics, the content in crude protein (CP: 26,1 %), which is comparable to that of leguminous hay, makes this plant suitable for animal nutrition [31] and for its high content in PUFA (70%) as well.

Hemp fatty acid profile (Table 2) is characterised by a great content in linoleic acid (C18:2 = 43.9 %), similar to that reported by Piovesana et al. [37]. This fatty acid is of particular interest in animal nutrition because it represents one of the main substrates of rumen bioydrogenation for CLAs synthesis [38]. Hemp cake and seeds, more used as supplement for animal nutrition [8,39,40], are similar to inflorescences in terms of LA content, whereas ALA result higher in the inflorescences.

The use of hemp showed increases in milk yield in other dairy ruminant when fed with hemp [39,40]. Nevertheless, the use of hemp inflorescences as dietary supplements did not determine changes in milk chemical composition, on the contrary to what observed by other authors which investigated on hemp seeds [41,42], where the milk protein and fat content significantly changed in treated group.

Nevertheless, nutritional characteristics of milks obtained by both groups were favorable to human health for their low omega-6/omega-3 ratio which is in line with what is suggested by nutritionists [43]. This result is probably due to the use of pasture in both groups as main source of forage, which is rich in omega-3 and provide a great quantity of other beneficial fatty acids in ruminants diet and in their derived products [44].

Lauric acid (C:12) significantly increased in GH group milk on the contrary of what observed by Rapetti et al. [8]. Lauric acid is a medium chain SFA which mainly derives by endogenous synthesis which still, can be inhibit by the abundance of long chain fatty acids in the diet [45]. In the last years, several authors have shown the numerous potentialities of lauric acid in in vitro studies [46–48], demonstrating its antidiabetic and anti-insuline resistence effects and its efficacy in inhibiting cancer cells proliferation.

With regards to the other milk fatty acids classes, other authors reported a significant increase in trans-vaccenic (C18:1 t11, VA), alfa-linolenic (C18:3, omega-3), eicosapentaenoic (EPA), docosahexaenoic (DHA) acids and in total CLA content [49] whereas a reduction in neo synthesis fatty acids (10:0-16:0) was observed [8,41] when different source of hemp are used as supplement. Among PUFA, we observed significantly higher content of linoleic acid ($p < 0,05$) and total CLA ($p < 0,0001$) in treated group. These increases are both explainable by the great content of omega-6 in hemp inflorescences which directly enriches the milk and at the same time works as substrate for rumen biohydrogenation increasing the total content of milk CLA isomers widely known for their anticancer properties as well as for the ability to fight inflammation and reduce the risk of cardiovascular diseases [11].

Of particular interest is the content of Adrenic Acid (C22:4), a long chain PUFA, which resulted significantly higher ($p < 0,001$) in GH group. The role of adrenic acid on human health is controversial; recent studies demonstrated its protective effect against the risk of depression [50] and its anti-inflammatory effects [51] while Zhao et al. [52] demonstrated its oxidative role in hepatocytes.

5. Conclusions

The aim of this study was to evaluate the effect of hemp inflorescences supplementation in the diet of grazing goats on milk yield and fatty acid profile. Our results showed that hemp supplementation affects milk fatty acid composition, increasing the total content of CLAs and of other beneficial FA, therefore suggesting that this plant could be a potential integration in animal diet

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useful to modify milk production, with advantages in terms of milk quality. Further studies are needed to evaluate different level of inflorescences supplementation and to investigates on aromatic characteristics of the product obtained. Moreover, the great attention that hemp had gained in the last years offers the possibility to use the different products that the cultivation of this plant offers in animal field.

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