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Camelina sativa (L. Crantz) Fresh Forage Productive Performance and Quality at Different Vegetative Stages: Effects of Dietary Supplementation in Ionica Goats on Milk Quality

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Abstract: The research meant to study the productive performances of *Camelina sativa* and the effects of feeding Camelina fresh forage harvested during five phenological stages (I: main stem elongation; II: maximum stem elongation: III: inflorescence appearance; IV: flowering; V: fruit set visible) on the yield, chemical composition and fatty acid profile of milk from autochthonous Ionica goats. Goats were randomly assigned to two groups (n = 15) that received a traditional forage mixture (Control) or Camelina forage harvested at different stages (CAM). The field experiment was conducted in two years; no significant differences between years were recorded for any of the Camelina production traits. The total biomass increased ($p < 0.05$) from phase I (1.4 t/ha) to phase V (5.2 t/ha). The distribution of stem, leaves and pod also changed during growth, showing a significant increase of stem from 40.8 to 45.6% and of pod from 0 to 19.4%, whereas leaves decreased from 59.2 to 35.1%. The milk yield and chemical composition were unaffected by the diet, while supplementation with Camelina forage increased milk CLA content (on average 1.14 vs. 0.78%). A markedly higher concentration of PUFAs was found in milk from goats fed Camelina harvested during the last three phenological stages. The index of thrombogenicity of milk from the CAM fed goats was significantly lower compared to the control group. In conclusion, *Camelina sativa* is a multi-purpose crop that may be successfully cultivated in Southern Italy regions and used as fresh forage for goat feeding. Milk obtained from Camelina fed goats showed satisfactory chemical and fatty acid composition, with potential benefits for human health.

Keywords: *Camelina sativa*; crop; phenological stage; forage; milk quality; fatty acids; Ionica goat

Citation: Tedone, L.; Giannico, F.; Tufarelli, V.; Laudadio, V.; Selvaggi, M.; De Mastro, G.; Colonna, M.A. *Camelina sativa* (L. Crantz) Fresh Forage Productive Performance and Quality at Different Vegetative Stages: Effects of Dietary Supplementation in Ionica Goats on Milk Quality. *Agriculture* **2022**, *12*, 91. https://doi.org/10.3390/agriculture12010091

Received: 6 December 2021
Accepted: 5 January 2022
Published: 11 January 2022

1. Introduction

Camelina (*Camelina sativa* L. Crantz), also known as false flax, is an oilseed crop belonging to the *Brassicaceae* family that can be grown as an annual summer or biannual winter crop [1]. Endowed with extreme rusticity and flexibility, the plant is well adapted to different climatic and soil conditions, with the exception of heavy clay and organic soil [2–6].

Camelina was extensively cultivated in the 19th century in France and, to a lesser ex-tent, in Holland, Belgium and Russia. Production gradually declined over time while recently the value of Camelina as an oilseed crop has been re-discovered, with the aim to promote crop diversification in European agricultural systems [7].

Camelina oil contains a high amount of Poly Unsaturated Fatty Acids (PUFA) that ranges from 65 to 67% of the total FA present in the plant during its growth cycle [8]; this concentration is greater in comparison to other common vegetable oils extracted from soybean, sunflower, rapeseed and olive [9,10]. Therefore, false flax has great food [11] and non-food potentials [12].

The agronomic interest towards Camelina is due to its adaptability to a wide range of environment temperatures; indeed, the plant resists to dry conditions [13] as well as to the cold winter climate [3]. Furthermore, the crop is characterized by low input production, that is influenced by the cultivar and time of sowing [14]. Matteo et al. [15] pointed out the opportunity to use Camelina in green chemistry applications under low input conditions.

The content and composition of Camelina seed oil may be variable [1]; these two fac-tors are taken into particular consideration in breeding programs [16]. Recent progress in breeding produced new varieties with high yield potential, improved quality of the seed and of the oil content and composition [7], resistance to lodging, shattering and to diseases. Other important aspects are the number of seeds per pod, the number of pods per plant, the growth cycle of the plant and the cultivation output applied.

Climate plays a strong influence on the FA composition of Camelina seeds, especially in terms of α-linolenic and linoleic acid concentration [4,10]. Moreover, the fatty acid profile changes during growing and filling of the seeds, with high contents of palmitic, stearic and oleic acid recorded especially during the first period [17].

An important prerequisite for the introduction of a new industrial oil crop relies on the ability of companies to identify new end-markets for their products [18,19] along with potential use of the residue of the oil-pressing process [20]. Oil and residues are strongly tied together, so that the success of an oil crop depends on the utilization of both products.

The production of oil by seed pressing provides an oil-cake as a by-product. Came-lina seed, oil and meal have been investigated for animal feeding in broilers [21,22], hens [23], pigs [24,25], rabbits [26,27], cows [28,29] and lambs [30], while little information is available on the use of Camelina as fresh forage.

The content of crude protein in the seed ranges from 25 to 45% [1,31]. According to earlier reports, the amount of crude fibre in Camelina seed is about 10%. The chemical composition of the seed depends on the varieties and is affected by the crop growth conditions.

A previous study carried out within the same research project focused the effects of dietary supplementation with Camelina fresh forage on the chemical, fatty acid composition and quality of milk and Caciotta cheese in Ionica goats. This autochthonous breed is well adapted to graze on arid soils and able to use the poor pastures of the Southern Italy regions. Mainly reared for milk production in small/medium agro-silvopastoral farms, the Ionica goat shows frugal feeding behavior that is very advantageous for farmers/breeders since it allows to reduce the feeding costs and to perform sustainable rearing systems [32].

The aim of the present research was to study the cultivation of *Camelina sativa* (L. Crantz) in a two-year field experiment carried out in a typical Southern Italy area. The productive performances and the chemical and fatty acid composition of the crop were investigated. Moreover, the effects of feeding Camelina fresh forage harvested during five different phenological stages (main stem elongation; maximum stem elongation: inflorescence appearance; flowering; fruit set visible) were studied on the yield, chemical composition and fatty acid profile of milk from multiparous goats of the autochthonous Ionica breed.

2. Materials and Methods

2.1. Field Experiments

A variety of false flax Camelina sativa (L. Crantz) named "Calena" was studied in a two-year field experiment (Y1: 2016/2017; Y2: 2017/2018) in a farm located in Gravina in Puglia (Apulia, Southern Italy, 40°59′42.22″ N, 16°20′38.82″ E, 345 m above the sea level). The autumn sowing dates in the two years were 13 October 2016 and 15 October 2017. The place chosen for the agronomic trial is representative of the climatical and soil conditions of

a typical Southern Italy internal area, characterized by low temperatures during winter and rainfall concentrated mainly during winter and spring (average annual rainfall of 450 mm).

Physical and chemical characteristics of the soil, performed in soil samples collected at 0–30 cm of depth, were analyzed at the beginning of the experiment in both years of cultivation. Soil organic matter was calculated using the Walkley-Black method [33]. Total nitrogen was evaluated using the macro-Kjeldahl digestion procedure [34] while available phosphorus was assessed by colorimetric analysis using the Olsen method [35]. Potassium content was determined according to the ammonium acetate method [36]. The data are reported in Table 1.

Table 1. Soil features (0–30 cm of depth) at the beginning of the experiment in the two test years.

Variable	Year	
	Y1 2016/2017	**Y2 2017/2018**
Sand (%)	41.1	43.2
Silt (%)	34.9	32.5
Clay (%)	24.0	24.3
pH	7.38	7.16
Organic Matter (%)	2.2	2.1
Total N g/kg	1.71	1.56
Available P (mg/kg)	21.7	23.1
Exchangeable K (mg/kg)	85.9	88.2

The trial was carried out by an "on farm" experiment, with a sowing surface of 1 ha of soil, previously sowed with durum wheat in both years of cultivation. The seeding rate, in each year, was approximately 6.5 kg/ha, taking into consideration percent seed germination (PSG) as well as 1000-seed weight (TSW), in order to reach a target of 500 plants/m^2. Sowing was performed by a seeding machine (La Valle Verde, Gravina in Puglia, Italy) with 0.18 m spaced rows and a depth of 0.01 m.

Soil preparation before sowing includes conventional deeper tillage with plowing during the summer period followed by harrowing before sowing. Pre-emergence herbicide containing Methazaclor was also applied.

Fertilization, in both experimental years, was performed using 57 kg P_2O_5/ha before sowing, as simple superphosphate, along with 63 kg N/ha, part of which was administered at sowing and later, in February, as ammonium sulphate.

The surface was divided into 20 plots of 500 m^2 each; biomass harvesting was scheduled beginning from stage 36 according to the BBCH scale (Biologische Bundesantalt, Bundessortenamt and Chemische Industrie) described by Martinelli and Galasso [37].

Five phenological growth stages of Camelina were taken into consideration:

I. main stem elongation (BBCH 36);
II. maximum stem elongation (BBCH 39);
III. inflorescence appearance (BBCH 55);
IV. flowering (BBCH 62);
V. fruit set visible (BBCH 69).

The crop was ripened on experimental areas of 20 m^2. During the different vegetative phases, the harvest was carried out cutting the whole vegetative part at 3 cm of height.

The accumulation of growing degree days (GDD) was calculated according to the formula: $GDD = \sum[(Tmax + Tmin)/2 - Tbase]$, where Tmax and Tmin are daily maximum and minimum air temperature and Tbase is the base temperature of 5 °C [15].

Weather data including air temperature and rainfall were collected at a permanent weather station located near the study site [38].

2.2. Camelina Biomass Analyses

The total biomass was weighed and the yield of the different vegetative parts was measured: stem, leaves and seeds. The biomass was desiccated at 65 °C until constant weight was recorded.

Fat and protein contents were assessed on representative samples from each plot. One sample of the biomass was used for lipid extraction [39]. The protein content was calculated by multiplying the total nitrogen percentage by 6.25 and total N content [40] was analysed using an Kjeltec 1030 instrument (Foss Tecator Analytical AB, Hoganas, Sweden).

The oil composition was measured using the transesterification method described by Lepage and Roy [41]. Fatty Acid Methyl Esters (FAME) were identified on a Shimadzu GC-14A (Kyoto, Japan) gas-liquid chromatography (GLC) equipped with flame ionization detector (FID) and C-R4AX chromatopack (Shimadzu, Kyoto, Japan) integrator. The carrier gas (helium) had a flow rate of 20 mL/min, split 1:40. Samples (1 µL) were injected onto a 30 m × 0.25 mm × 0.2 µm film thickness Supelco SP™ 2380 (Bellefonte, PA, USA) capillary column. The injector and FID temperature was 250 °C. The initial column temperature was 100 °C. The temperature program increased by 5 °C/min to 175 °C. After 10 min at 175 °C, the temperature increased by 8 °C/min to 220 °C and then was kept for 10 min at 220 °C. A comparison between the retention times of the samples with those of co-injected authentic standards was made to facilitate identification. Column chromatography (CC) separation of total lipid (TL) extracts in chloroform was separated into normal lipids (NL), glycolipids (GL) and phospholipids (PL) by passing through a glass column (diameter: 20 mm, length: 30 cm) packed with a slurry of activated silicic acid (70/230 mesh; Merck, Darmstadt, Germany) in chloroform (1:5, *w/v*) according to Rouser et al. [42]. The eluting solvents for NL, GL, and PL were chloroform, acetone and methanol, respectively. Solvents were evaporated by using a rotary evaporator, and the percentage of each fraction was gravimetrically determined.

The potential nutritive value and in vitro gas production (IVGP) parameters of Camelina were assessed according to the method of Menke et al. [43] as previously described [33]. The gas production (GP) was expressed in ml/g Dry Matter (DM), while the metabolizable energy (MJ/kg DM) was calculated using the formula: ME = 1.06 + 0.157GP + 0.084CP + 0.22CF − 0.081A, in which GP is the net gas production in 72 h (mL/g DM) and CP, CF and A are the values of crude protein, crude fat and ash (% DM), respectively.

2.3. Animals, Diet, Management and Milk Sampling

Thirty female multiparous goats of the autochthonous Ionica breed, homogeneous for weight (48 ± 2 kg), parity (3–4), time of kidding (30 ± 5 days after delivery) and milk yield (1.80 ± 0.2 kg/day), were randomly assigned to two groups (n = 15) that differed between each other only for the type of forage administered: the control group (C) received a traditional forage mixture (*Avena sativa*, 70%; *Vicia sativa*, 20%; *Trifolium* spp., 10%), while the experimental group (CAM) was given Camelina sativa fresh forage harvested at five different phenological stages (I–V). According to the traditional goat rearing system, during the day all the dams grazed on a spontaneous vegetation typical of the Mediterranean area; at housing, in the evening, the dams of the two groups received the same commercial feed (500 g/head/day) along with one of the two types of forage [32].

The experimental period lasted 2 months. During each phenological stage of development of Camelina, milk samples were collected twice, 1 week apart, for a total of 10 samplings. Milk was collected from control goats during the same sampling days as de-scribed for Camelina fed goats, in order to study milk yield and quality evolution in the correspondent periods. Therefore, milk samplings report the same labelling used for phenological stages of development of Camelina, i.e., I, II, III, IV and V, respectively.

Individual milk samples were collected twice a day (7.00 a.m. and 6.00 p.m.); they were weighed, mixed together and stored at +4 °C. Milk collected per each dam was divided into two aliquots, one of which was analyzed for fat, protein and lactose by an infrared milk analyzer (Milkoscan 133-B, Foss Electric, Hillerod, Denmark) previously standardized

for goat milk, while the second aliquot was stored at −80°C until fatty acid analysis was performed. Somatic cells count (SCC) was also assessed and the result (10^3/mL) was transformed into logarithmic form (log10).

Pasture samples were collected along transects according to the methods previously described [44]. Samples of grass and Camelina sativa green biomass were dried at 60°C for 48 h in a stove, homogenized and analyzed. Samples of the pelleted feed, dried pasture, hay and Camelina sativa forage were ground in a hammer mill through a 1 mm sieve and analyzed using the Association of Official Analytical Chemists (AOAC) procedures [40]: DM (method 934.01), fat (method 920.39), ash (method 942.05), protein (method 954.01), crude fibre (method 945.18), ADF (Acid Detergent Fibre) and ADL (Acid Detergent Lignin) (method 973.18) and NDF (Neutral Detergent Fibre, method 2002.04).

2.4. Fatty Acid Profiles of Feeds and Milk

Total lipids were extracted from the homogenized samples (100 g) according to the chloroform and methanol method described by Folch et al. [45]. Fatty acids (FA) were methylated by using a BF_3-methanol solution (12% *v/v*) [46]. The FA profile was assessed with a Chrompack CP 9000 gas chromatograph with a silicate glass capillary column (70% cyanopropyl polysilphenylene-siloxane BPX 70 of SGE Analytical Science, Chebios S.r.l., Rome, Italy; length: 50 m; internal diameter: 0.22 mm; film thickness: 0.25 µm). The temperature program was 135 °C for 7 min, followed by increases of 4 °C per minute up to 210 °C. Fatty acid peaks were identified by using a comparative analysis with standard reference mixtures. For the identification of C18:2 cis9,trans11 isomer (Conjugated Linoleic Acid, CLA), a mixture of CLA methyl esters was used (Sigma-Aldrich, Milan, Italy).

2.5. Statistical Analysis

Statistical analysis was performed using the SAS statistical software application [47]. Data referring to Camelina growth and productive performances were processed taking into consideration year (Y) and phenological stage (S) as main effects, along with their interaction (YxS). As for statistical processing of milk production and quality traits, the main effects were year (Y), phenological stage (S), diet (D) and their interactions (YxS, YxD, SxD, YxSxD). However, since no significant effect of year was observed neither for Camelina traits nor for milk yield and quality, the fixed effect of year was excluded from the model.

Comparison among the levels of dependent variables was achieved by Tukey's HSD test at $p < 0.05$. Data are presented as least squares means ± standard error.

3. Results

3.1. Weather Trend

Weather conditions recorded during the trial period are shown in Figure 1. The Bagnouls-Gaussen diagram shows the thermo-pluviometric annual variations (Y1 and Y2) occurred in the test place in comparison with long-term average (1980–2015) rainfall and maximum and minimum temperatures. The meteorological data recorded during the two test years confirmed the typical trend of the Mediterranean climate, namely the occurrence of an arid period during the summer months (June–September) with average temperatures around 25 °C, and winter-spring period characterized by mild climate, with temperatures which rarely dropped below 0 °C. Average rainfall was about 500 mm/year, concentrated mainly between November and March.

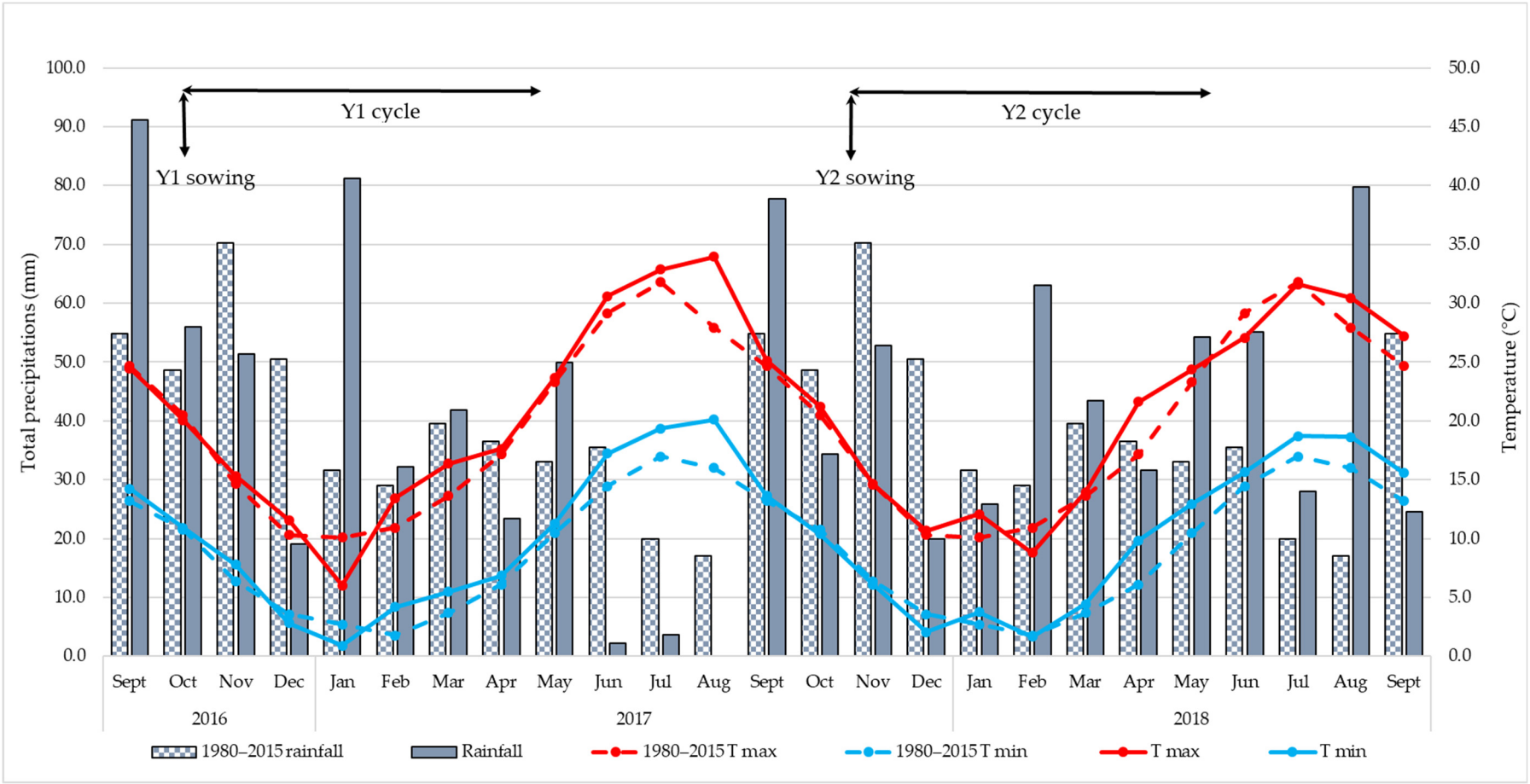

Figure 1. Monthly precipitations and mean minimum and maximum air temperatures (°C) during the two-year study period (Y1: 2016–2017 and Y2: 2017–2018) compared to average long-term (1980–2015) values. Chart legend: Dotted histogram and lines indicate long-term (1980–2015) rainfall (dotted grey) and temperatures (maximum: dotted red; minimum: dotted light blue). Solid coloured histogram and lines indicate rainfall (grey) and temperatures (maximum: red; minimum: light blue) during the study period. Vertical arrows show the sowing dates (Y1: October 2016; Y2: October 2017); horizontal double-headed arrows indicate the length of the crop cycle in the two test years.

During 2016/2017 (Y1), temperatures were above the average, with exception for January 2017. A similar trend was recorded also for 2017/2018 (Y2), in which during November–December 2017 and in February 2018 temperatures below the average were registered.

As for precipitations, rainfall during both test years was comparable to the long-term average, except for the range June–August 2017 that was particularly dry; however, this period may not have affected the Camelina growth cycle because it falls in the middle of two consecutive crops. The two-years rainfall average from September to May was 393 mm; in particular, the rainfall measured during 2016/2017 and 2017/2018 was 426 and 423 mm, respectively. In general, although the rainfall presented an irregular distribution over time, the water requirement of the Camelina crop was adequately fulfilled in both test years.

3.2. Camelina Phenological Stages

The duration of the Camelina growth cycle is shown in Figure 2. The crop showed an average length of about 224 and 215 days, respectively during the first and second test year. The different phases had, on average, the following lengths: 7 days, sowing—emergence (00–09); 33 days, emergence—leaf development (09–15); 79 days, leaf development—main shoot elongation (15–31); 42 days, main stem elongation—inflorescence appearance (31–51); 19 days, inflorescence appearance—start flowering (51–0); 11 days, flowering—fruit set visible (60–64); 19 days, fruit set visible- physiological maturation (64–79); 10 days, physiological maturation—harvesting maturation (79–89).

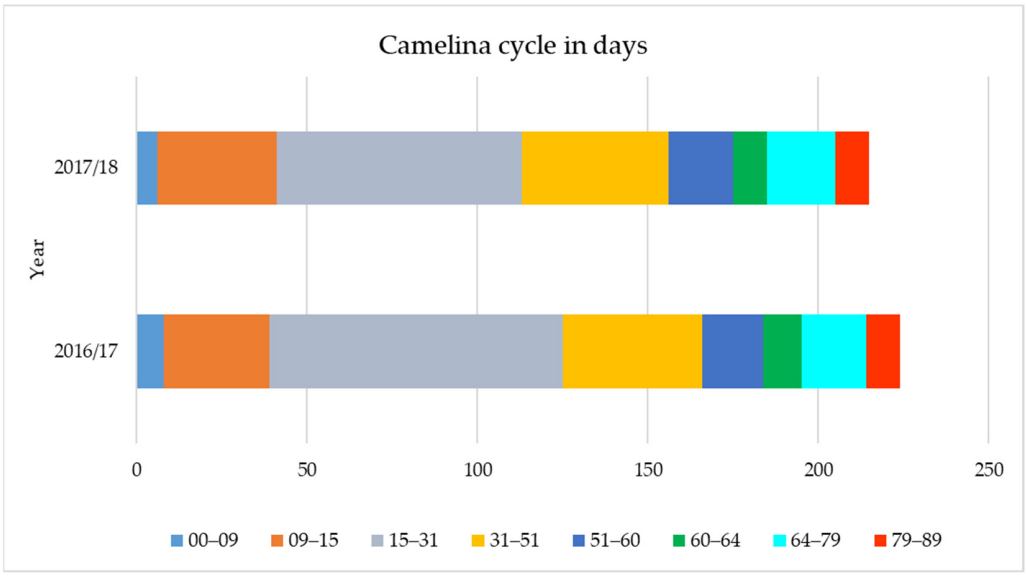

Figure 2. Duration in days of the main phenological growth stages of Camelina sowed in 2016–2017 (Y1) and 2017–2018 (Y2).

The results regarding the growing degree days (GDD) measures are shown in Table 2. The complete cycle required 1208 GDD during the first year, while 1147 in the second one. The main difference between the two years was for the vegetative period, that was 80 GDD greater in 2016/2017 as compared to 2017/2018. This may probably be linked to the cumulative rain measured during the two years, since the total rainfall was greater during 2016/2017.

Table 2. Growing degree days (GDD) of the main phenological growth stages of *Camelina sativa* sowed in autumn 2016/2017 (Y1) and 2017/2018 (Y2).

Phenological Growth Stages	Length (Days)	Year	
		Y1 2016/2017	Y2 2017/2018
Sowing-emergence	00–09	87.2	96.9
Emergence-leaf development	09–15	247.7	228.3
Leaf development-stem elongation	15–31	106.8	121.6
Stem elongation-inflorescence visible	31–51	209.7	165.2
Inflorescence appearance-start flowering	51–60	148.7	121.6
Start flowering-fruit set visible	60–64	70.0	91.2
Fruit set visible-physiological maturation	64–79	208.4	210.8
Physiological maturation-harvest maturation	79–89	129.9	111.6
Total		1208.3	1147.1

3.3. Camelina Biometric, Biomass Production and Quality Traits

Time course of the plant height is shown in Figure 3. The plant height progressively increased from the stem elongation (average 23.4 cm) to the fruit set visible phase (average 50.2 cm). Significant differences ($p < 0.05$) aroused, in particular, between the first two phenological stages, i.e., from main to maximum stem elongation.

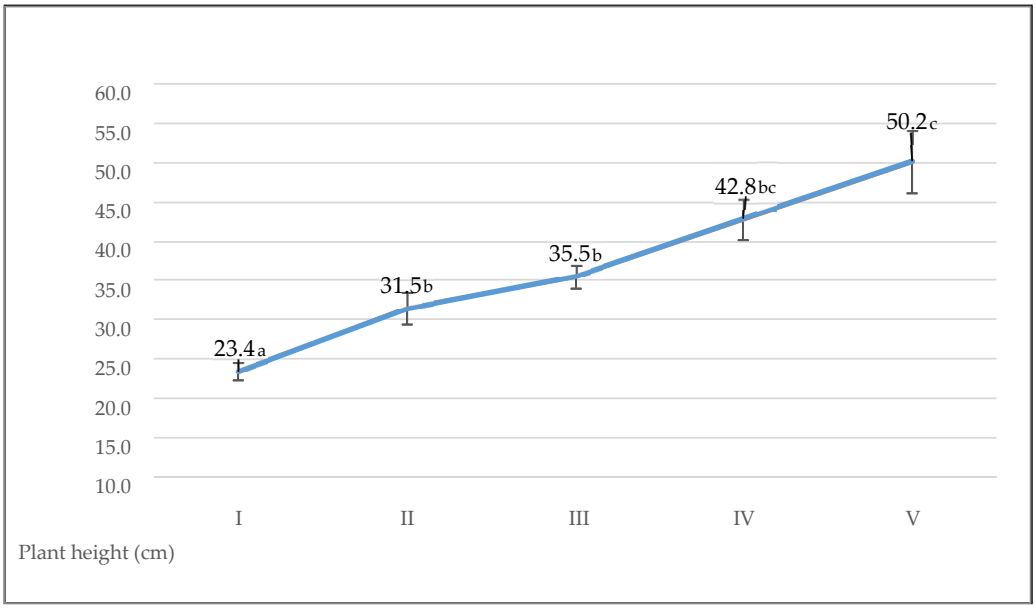

Figure 3. Camelina sativa plant height evolution (average between the two test years). I: main stem elongation; II: maximum stem elongation: III: inflorescence appearance; IV: flowering; V: fruit set visible. Means with different lower-case letters are significantly different at $p < 0.05$.

Results referring to Camelina production traits are shown in Table 3. The total fresh biomass production increased ($p < 0.05$) from phase I (6.7 t/ha) to phase V (19.0 t/ha). Similarly, the dry biomass production increased ($p < 0.05$) from 1.4 to 5.2 t/ha. The distribution of stem, leaves and pod also widely changed during growth, showing a significant increase ($p < 0.05$) of stem from 40.8 to 45.6% and of pod from 0 to 19.4% ($p < 0.05$), while leaves decreased from 59.2 to 35.1% ($p < 0.05$).

Table 3. Productive performances of *Camelina sativa* harvested during phenological stages (average between the two test years) (mean ± standard error).

Trait	Phenological Stages [1]				
	I BBCH [2] 36	II BBCH 39	III BBCH 55	IV BBCH 62	V BBCH 69
Fresh biomass production (ton/ha)	6.7 ± 0.44 [c]	11.0 ± 0.22 [b]	12.6 ± 0.32 [b]	17.6 ± 1.53 [a,b]	19.0 ± 0.95 [a]
Dry biomass production (ton/ha)	1.4 ± 0.08 [c]	2.5 ± 0.09 [b]	2.9 ± 0.06 [b]	4.0 ± 0.41 [ab]	5.2 ± 0.40 [a]
Distribution of production (%)					
- stem	40.8 ± 0.87 [b]	42.0 ± 0.42 [b]	43.9 ± 1.24 [a,b]	44.9 ± 0.42 [a,b]	45.6 ± 0.88 [a]
- leaves	59.2 ± 0.77 [a]	58.0 ± 0.53 [a]	56.1 ± 0.82 [a]	48.4 ± 0.78 [b]	35.1 ± 0.77 [c]
- pod	0.0 [c]	0.0 [c]	0.0 [c]	6.7 ± 0.78 [b]	19.4 ± 0.40 [a]

[1] I: main stem elongation; II: maximum stem elongation: III: inflorescence appearance; IV: flowering; V: fruit set visible. [2] BBCH: Biologische Bundesantalt, Bundessortenamt and Chemische Industrie. On the row: means with different lower-case letters are significantly different at $p < 0.05$.

Results regarding the chemical composition and nutritive value of Camelina harvested during the different growth stages are reported in Table 4. The protein content was markedly higher during the first three phases of growth ($p < 0.05$), with values between 16.55 and 17.10%, while it progressively lowered during the phases of flowering and fruit set visible (14.7 and 14.4%, respectively).

Table 4. Chemical composition and nutritive value of Camelina harvested during different phenological stages (mean ± standard error).

	Camelina Phenological Stages [1]				
	I BBCH [2] 36	II BBCH 39	III BBCH 55	IV BBCH 62	V BBCH 69
Dry matter (DM, %)	20.15 ± 0.31 [c]	22.35 ± 0.57 [b]	23.20 ± 0.43 [b]	22.95 ± 0.42 [b]	27.35 ± 0.68 [a]
Protein	16.80 ± 0.45 [a]	16.55 ± 0.60 [a]	17.10 ± 0.16 [a]	14.7 ± 0.57 [b]	14.4 ± 0.47 [b]
Fat	3.40 ± 0.08	3.25 ± 0.12	3.30 ± 0.03	3.20 ± 0.10	3.20 ± 0.17
Ash	8.91 ± 0.41 [a]	8.61 ± 0.31 [a,b]	8.45 ± 0.12 [b]	7.55 ± 0.08 [c]	8.94 ± 0.08 [a]
GP [3] (mL/g DM)	181.60 ± 9.32 [a,b]	172.55 ± 8.58 [b]	175.95 ± 3.82 [b]	180.93 ± 4.93 [a,b]	199.16 ± 2.99 [a]
ME [4] (MJ/kg DM)	8.25 ± 0.21 [a,b]	8.35 ± 0.21 [a]	7.91 ± 0.13 [a,b]	7.77 ± 0.21 [b]	8.40 ± 0.07 [a]

[1] I: main stem elongation; II: maximum stem elongation: III: inflorescence appearance; IV: flowering; V: fruit set visible. [2] BBCH: Biologische Bundesantalt, Bundessortenamt and Chemische Industrie. [3] In vitro gas production; [4] Metabolizable energy. Means with different lower-case letters in each row are significantly different at $p < 0.05$.

No significant changes in the fat content of the plant were recorded during growth, with values falling between 3.20 and 3.40%. The lowest content in ash was found during the flowering stage of Camelina (7.55%), with marked differences ($p < 0.05$) in comparison with the other phenological stages (range 8.45–8.94%).

Evaluation of the nutritive value of Camelina forage by the IVGP method showed that the highest values in terms of gas production ($p < 0.05$) were recorded during the fruit set visible stage (V; 199.16 mL/g DM). The metabolizable energy of Camelina forage was lowest in correspondence of the flowering phase (IV; 7.77 MJ/kg DM) while highest ($p < 0.05$) during the stages of maximum stem elongation (II; 8.35 MJ/kg DM) and fruit set visible (V; 8.40 MJ/kg DM).

Table 5 reports the fatty acid profile of Camelina forage harvested during the different phenological stages. Some fatty acids showed similar patterns in terms of an increase or decrease in concentration during Camelina growth. In particular, the saturated myristic acid markedly decreased during the last stage (0.31 vs. 0.56–0.62%). Palmitic, stearic and arachidic fatty acids showed a progressive decrease over time in turn of an increase of the concentration of linoleic and α-linolenic acids, as well as of gondoic acid that was highest during the fruit set visible stage ($p < 0.05$). The concentration of oleic and behenic acids was quite steady across the first three phenological stages. The content of oleic acid significantly

($p < 0.05$) increased from flowering onwards while behenic acid showed a significant ($p < 0.05$) peak during flowering. The amount of erucic acid was low during the beginning of Camelina growth while it increased during the last stage ($p < 0.05$), as expected.

Table 5. Major fatty acids (% of Fatty Acid Methyl Esters, FAME) in Camelina fresh forage harvested during different phenological stages (mean ± standard error).

Fatty Acid (% FAME)	Camelina Phenological Stages [1]				
	I BBCH[2] 36	II BBCH 39	III BBCH 55	IV BBCH 62	V BBCH 69
$C_{14:0}$, myristic	0.56 ± 0.03 ^{a}	0.61 ± 0.02 ^{a}	0.60 ± 0.01 ^{a}	0.62 ± 0.04 ^{a}	0.31 ± 0.05 ^{b}
$C_{16:0}$, palmitic	20.69 ± 0.20 ^{a}	19.97 ± 0.22 ^{a,b}	19.48 ± 0.33 ^{b}	17.29 ± 0.21 ^{c}	14.10 ± 0.28 ^{d}
$C_{18:0}$, stearic	18.62 ± 0.15 ^{a}	17.74 ± 0.18 ^{b}	16.95 ± 0.20 ^{b}	15.21 ± 0.35 ^{c}	11.78 ± 0.45 ^{d}
$C_{18:1n9cis}$, oleic	16.40 ± 0.23 ^{b}	16.59 ± 0.13 ^{b}	16.65 ± 0.18 ^{b}	17.23 ± 0.25 ^{a}	17.61 ± 0.40 ^{a}
$C_{18:2n6}$, linoleic	18.34 ± 0.26 ^{c}	19.04 ± 0.24 ^{c}	20.22 ± 0.15 ^{b}	20.77 ± 0.31 ^{b}	22.45 ± 0.43 ^{a}
$C_{18:3n3}$, α-linolenic	4.87 ± 0.23 ^{e}	6.17 ± 0.70 ^{d}	7.72 ± 0.14 ^{c}	10.91 ± 0.18 ^{b}	13.34 ± 0.33 ^{a}
$C_{20:0}$, arachidic	2.70 ± 0.18 ^{a,b}	2.96 ± 0.27 ^{a}	2.72 ± 0.14 ^{a,b}	2.26 ± 0.09 ^{b}	1.38 ± 0.06 ^{c}
$C_{20:1}$, gondoic	0.16 ± 0.02 ^{b}	0.17 ± 0.04 ^{b}	0.19 ± 0.02 ^{b}	0.40 ± 0.02 ^{b}	2.15 ± 0.23 ^{a}
$C_{22:0}$, behenic	0.64 ± 0.02 ^{b}	0.63 ± 0.04 ^{b}	0.52 ± 0.10 ^{b}	1.01 ± 0.17 ^{a}	0.56 ± 0.06 ^{b}
$C_{22:1}$, erucic	0.00 ± 0.00 ^{c}	0.00 ± 0.00 ^{c}	0.04 ± 0.02 ^{b}	0.04 ± 0.02 ^{b,c}	0.12 ± 0.01 ^{a}

[1] I: main stem elongation; II: maximum stem elongation: III: inflorescence appearance; IV: flowering; V: fruit set visible. [2] BBCH: Biologische Bundesantalt, Bundessortenamt and Chemische Industrie. Means with different lower-case letters in each row are significantly different at $p < 0.05$.

3.4. Goat Milk Yield, Chemical and Fatty Acid Composition

The yield and chemical composition of milk obtained from goats fed a control forage mixture or Camelina fresh forage is reported in Table 6. No differences between dietary treatments aroused for any of the milk variables. In both groups, the somatic cell count (SCC) gradually increased over time, reaching significantly ($p < 0.05$) higher values during the interval IV-V in comparison with stages I-III. Moreover, a greater SCC value was recorded in control milk samples as compared to the CAM group ($p < 0.05$) in the last two stages.

Table 6. Chemical composition (mean ± standard error) of goat milk collected during different phenological stages of Camelina.

		Milk Samplings during Camelina Phenological Stages [1]					Effects [2]		
		I	II	III	IV	V	S	D	SxD
Milk yield (kg/day)	Control	2.05 ± 0.25	1.95 ± 0.26	1.90 ± 0.18	1.80 ± 0.15	1.75 ± 0.15	ns	ns	ns
	Camelina	2.01 ± 0.25	1.97 ± 0.26	1.92 ± 0.18	1.88 ± 0.15	1.78 ± 0.15			
Dry Matter (% DM)	Control	13.37 ± 0.53	12.53 ± 0.41	13.41 ± 0.48	12.97 ± 0.49	12.97 ± 0.38	ns	ns	ns
	Camelina	13.46 ± 0.60	12.34 ± 0.43	12.84 ± 0.46	12.54 ± 0.40	12.65 ± 0.50			
Fat	Control	4.36 ± 0.34	3.98 ± 0.30	4.17 ± 0.34	3.92 ± 0.29	3.92 ± 0.23	ns	ns	ns
	Camelina	4.26 ± 0.58	3.92 ± 0.33	4.01 ± 0.35	3.98 ± 0.33	4.01 ± 0.30			
Protein	Control	3.64 ± 0.18	3.64 ± 0.15	3.69 ± 0.17	3.87 ± 0.19	3.67 ± 0.14	ns	ns	ns
	Camelina	3.55 ± 0.14	3.60 ± 0.09	3.43 ± 0.20	3.60 ± 0.13	3.64 ± 0.15			
Lactose	Control	4.62 ± 0.07	4.54 ± 0.07	4.60 ± 0.06	4.55 ± 0.07	4.56 ± 0.05	ns	ns	ns
	Camelina	4.57 ± 0.07	4.57 ± 0.05	4.55 ± 0.04	4.56 ± 0.05	4.52 ± 0.05			
Somatic cells (log10)	Control	1.19 ± 0.06 ^{b}	1.29 ± 0.04 ^{b}	1.35 ± 0.02 ^{b}	1.74 ± 0.09 ^{a*}	1.70 ± 0.03 ^{a*}	*	*	ns
	Camelina	1.03 ± 0.04 ^{b}	1.09 ± 0.04 ^{b}	1.12 ± 0.02 ^{b}	1.45 ± 0.03 ^{a}	1.42 ± 0.04 ^{a}			

[1] I: main stem elongation; II: maximum stem elongation: III: inflorescence appearance; IV: flowering; V: fruit set visible. [2] S: phenological stage; D: diet; * $p < 0.05$; ns: not significant. On the row: means with different lower-case letters are significantly different at $p < 0.05$. On the column: * indicates means significantly different at $p < 0.05$; ns = not significant.

The results concerning the fatty acid profile of goat milk are shown in Table 7. In both groups, the highest ($p < 0.05$) concentration of oleic acid ($C_{18:1}$) was recorded during the last two stages. The content of linoleic acid ($C_{18:2\ n\text{-}6}$) tended to increase over time in the CAM group; as a matter of fact, the concentration of this fatty acid during the fruit set visible stage (V) was significantly ($p < 0.05$) greater in comparison to the first two stem elongation stages (I-II). In both groups, evolution of the concentration of conjugated linoleic acid (CLA) displayed a similar trend, i.e., a gradual increase during stages I-II which reached the highest values in correspondence of stage III followed by a significant ($p < 0.05$) decrease in the last two stages. Furthermore, except for the first stage, milk from goats fed Camelina forage showed a significantly ($p < 0.05$) greater CLA concentration in comparison with the control group, especially during stage III ($p < 0.01$). The concentration of α-linolenic acid ($C_{18:3\ n\text{-}3}$) did not show any difference in the control milk samples, while in goats fed Camelina forage, the highest level of α-linolenic in milk was recorded during stage V, with significant differences also in comparison with the control group ($p < 0.05$). The concentration of arachidonic acid ($C_{20:4}$) was significantly ($p < 0.05$) higher during stage V in comparison with the other stages of the CAM group as well as compared to the control group during the same stage. The concentration of the polyunsaturated acids EPA and DHA displayed a similar trend in both groups: the highest concentration was found in stages IV-V, with significant differences ($p < 0.05$) in comparison with the other ones. Moreover, the EPA concentration was greater ($p < 0.05$) in the CAM group during stages IV-V as compared to the control one.

Milk collected from goats fed with Camelina forage harvested from stages III to V showed a lower concentration of total SFA as compared to control samples, although not at a significant level. No differences in terms of concentration of MUFAs were found, neither between groups nor among stages. With exception for stage I, the content of PUFA in milk samples from the CAM fed group was always greater ($p < 0.05$) in comparison with the control one. Furthermore, the highest level of PUFA was found in milk from goats fed Camelina forage harvested during the fruit set visible stage ($p < 0.05$).

Among PUFAs, the main differences between diets aroused for the total n-3 fatty acids, whose concentration in milk samples of the CAM group was greater compared to the control ones from stage II to V ($p < 0.05$). Moreover, during stage V, the content of n-6 fatty acids was markedly ($p < 0.05$) higher in the CAM group as compared to the control one. As a consequence, the n-6/n-3 ratio in CAM milk samples was lower within the interval II-V ($p < 0.05$).

The index of atherogenicity was significantly lower ($p < 0.05$) in the CAM group only in correspondence of stage V, whereas a significant difference between groups was observed for the index of thrombogenicity (TI), that was markedly lower ($p < 0.05$) in milk samples collected from goats fed Camelina forage.

Table 7. Main fatty acid composition (% of Fatty Acid Methyl Esters, FAME) of goat milk collected during different phenological stages of Camelina (mean ± standard error).

Fatty Acid (% FAME)	Diet	Milk Samplings during Camelina Phenological Stages [1]					Effects [2]		
		I	II	III	IV	V	S	D	SxD
$C_{18:1n9cis}$, oleic	Control	17.75 ± 0.14 ^{a,b}	17.57 ± 0.07 ^{b}	17.32 ± 0.27 ^{b}	18.07 ± 0.200 ^{a}	18.34 ± 0.25 ^{a}	*	ns	ns
		ns	ns	ns	ns	ns			
	Camelina	17.59 ± 0.11 ^{c}	17.63 ± 0.01 ^{c}	18.06 ± 0.24 ^{b,c}	18.99 ± 0.27 ^{b}	19.87 ± 0.14 ^{a}			
$C_{18:2n6}$, linoleic	Control	2.28 ± 0.02	2.28 ± 0.03	1.97 ± 0.07	2.27 ± 0.03	2.13 ± 0.03	*	ns	ns
		ns	ns	ns	ns	ns			
	Camelina	2.34 ± 0.02 ^{b}	2.39 ± 0.04 ^{b}	2.42 ± 0.13 ^{a,b}	2.54 ± 0.03 ^{a,b}	2.68 ± 0.02 ^{a}			
$C_{18:2c9,t11}$, CLA	Control	0.65 ± 0.02 ^{b,c}	0.95 ± 0.01 ^{a}	0.98 ± 0.03 ^{a}	0.77 ± 0.01 ^{b}	0.55 ± 0.01 ^{c}	*	*	ns
		ns	*	**	*	*			
	Camelina	0.65 ± 0.02 ^{d}	1.33 ± 0.01 ^{b}	1.95 ± 0.11 ^{a}	0.95 ± 0.03 ^{c}	0.84 ± 0.01 ^{c,d}			
$C_{18:3n3}$, α-linolenic	Control	0.31 ± 0.00	0.35 ± 0.00	0.29 ± 0.02	0.38 ± 0.00	0.30 ± 0.00	*	ns	ns
		ns	ns	ns	ns	*			
	Camelina	0.34 ± 0.00 ^{b}	0.29 ± 0.01 ^{b}	0.30 ± 0.01 ^{b}	0.42 ± 0.01 ^{a,b}	0.47 ± 0.00 ^{a}			
$C_{20:4}$, arachidonic	Control	0.28 ± 0.00	0.19 ± 0.00	0.24 ± 0.01	0.25 ± 0.00	0.26 ± 0.00	*	ns	ns
		ns	ns	ns	ns	*			
	Camelina	0.34 ± 0.00 ^{b,c}	0.29 ± 0.01 ^{c}	0.30 ± 0.01 ^{b,c}	0.42 ± 0.00 ^{b}	0.47 ± 0.00 ^{a}			
$C_{20:5\ n-3}$, EPA	Control	0.11 ± 0.00 ^{b}	0.13 ± 0.00 ^{b}	0.13 ± 0.01 ^{b}	0.17 ± 0.00 ^{a}	0.18 ± 0.00 ^{a}	*	*	ns
		ns	ns	ns	*	*			
	Camelina	0.14 ± 0.00 ^{b}	0.16 ± 0.00 ^{b}	0.18 ± 0.01 ^{b}	0.23 ± 0.00 ^{a}	0.25 ± 0.00 ^{a}			
$C_{22:6\ n-3}$, DHA	Control	0.04 ± 0.00 ^{b}	0.04 ± 0.00 ^{b}	0.03 ± 0.01 ^{b}	0.08 ± 0.00 ^{a}	0.08 ± 0.00 ^{a}	*	ns	ns
		ns	ns	ns	ns	ns			
	Camelina	0.04 ± 0.00 ^{b}	0.05 ± 0.00 ^{b}	0.05 ± 0.00 ^{b}	0.08 ± 0.00 ^{b}	0.08 ± 0.00 ^{a}			
Total SFA	Control	74.05 ± 0.09	74.91 ± 0.05	76.02 ± 0.36	75.23 ± 0.10	74.51 ± 0.25	ns	ns	ns
		ns	ns	ns	ns	ns			
	Camelina	73.56 ± 0.06	73.58 ± 0.02	74.17 ± 0.40	73.77 ± 0.12	73.66 ± 0.09			
Total MUFA	Control	21.62 ± 0.08	20.89 ± 0.07	19.83 ± 0.32	20.57 ± 0.15	21.25 ± 0.30	ns	ns	ns
		ns	ns	ns	ns	ns			
	Camelina	21.96 ± 0.03	21.68 ± 0.07	20.83 ± 0.21	21.23 ± 0.10	20.70 ± 0.07			
Total PUFA	Control	4.33 ± 0.02	4.20 ± 0.02	4.14 ± 0.13	4.20 ± 0.05	4.24 ± 0.04	*	*	ns
		ns	*	*	*	*			
	Camelina	4.48 ± 0.03 ^{c}	4.74 ± 0.05 ^{c}	5.00 ± 0.19 ^{b}	5.00 ± 0.02 ^{b}	5.64 ± 0.02 ^{a}			
PUFA/SFA	Control	0.06 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	ns	ns	ns
		ns	ns	ns	ns	ns			
	Camelina	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.00			
Total n-6	Control	3.71 ± 0.02	3.71 ± 0.02	3.63 ± 0.11	3.41 ± 0.04	3.21 ± 0.04	*	ns	ns
		ns	ns	ns	ns	*			
	Camelina	3.85 ± 0.03 ^{a}	3.94 ± 0.04 ^{a}	3.58 ± 0.19 ^{b}	3.29 ± 0.01 ^{c}	3.75 ± 0.01 ^{b}			
Total n-3	Control	0.62 ± 0.00 ^{a,b}	0.48 ± 0.00 ^{b}	0.52 ± 0.00 ^{b}	0.79 ± 0.00 ^{a,b}	0.83 ± 0.00 ^{a}	*	*	ns
		ns	*	*	*	*			
	Camelina	0.63 ± 0.00 ^{d}	0.81 ± 0.01 ^{d}	1.42 ± 0.01 ^{c}	1.71 ± 0.00 ^{b}	1.89 ± 0.00 ^{a}			
n-6/n-3	Control	5.98 ± 0.00 ^{c}	7.73 ± 0.00 ^{a}	6.98 ± 0.04 ^{b}	4.32 ± 0.00 ^{d}	3.86 ± 0.00 ^{e}	*	*	*
		ns	*	*	*	*			
	Camelina	6.11 ± 0.00 ^{a}	4.86 ± 0.00 ^{b}	2.52 ± 0.13 ^{c}	1.92 ± 0.00 ^{d}	1.98 ± 0.00 ^{d}			
AI	Control	2.80 ± 0.01	2.77 ± 0.03	2.65 ± 0.08	2.59 ± 0.01	2.77 ± 0.05	ns	*	ns
		ns	ns	ns	ns	*			
	Camelina	2.75 ± 0.01 ^{d}	2.54 ± 0.02 ^{b,c}	2.40 ± 0.09 ^{b,c}	2.30 ± 0.02 ^{b,c}	2.21 ± 0.01 ^{a}			
TI	Control	3.32 ±0.00 ^{c}	3.25 ± 0.03 ^{c}	3.23 ± 0.08 ^{a}	3.29 ± 0.01 ^{a,b}	3.20 ± 0.05 ^{b}	*	*	ns
		*	*	*	*	*			
	Camelina	3.05 ±0.01 ^{b}	2.80 ± 0.02 ^{a}	2.59 ± 0.12 ^{a}	2.33 ± 0.00 ^{a}	2.14 ± 0.01 ^{a}			

[1] I: main stem elongation; II: maximum stem elongation: III: inflorescence appearance; IV: flowering; V: fruit set visible. [2] S: phenological stage; D: diet; * $p < 0.05$; ns: not significant. On the row: means with different lower-case letters are significantly different at $p < 0.05$. On the column: * indicates means significantly different at $p < 0.05$; ns = not significant.

4. Discussion

4.1. Camelina Biometric, Biomass Production and Quality Traits

Camelina sativa is becoming an interesting cultivation in European countries due to its multi-purpose uses in food, feed and biobased applications [4,5,7,11,19]. Under an agronomic point of view, the plant is flexible and shows a fast growth cycle [7]. This feature enables the use of Camelina as a cover crop that can be easily turned into a cash cover crop [15].

False flax can also be used in mixed crops, especially in environmental limitation conditions. Several experiences showed that Camelina can be successfully grown in association with peas (*Pisum sativum* L.), lentils (*Lens culinaris* L.) and lupins (*Lupinus angustifolius* L. or *Lupinus album* L.) [7], that are legume crops commonly cultivated in Southern Italy regions.

In this study, the chemical properties and nutritive value of the plant changed during its cycle. In particular, the false flax forage showed the best nutritive value, during the fruit set visible stage, both in terms of in vitro gas production and calculated metabolizable energy. Peiretti and Meineri [7] found a decrease of the in vitro organic matter digestibility of the Camelina whole plant during growth, due to the progressive effect of lignification. The morphological development of the plant widely affects the nutritive value of several grass and legume forages; therefore, it is important to study the evolution of the chemical composition and digestibility in order to harvest the crop at the optimum stage, thus reducing the effects of maturity and the loss of nutritive value of the forage.

Oil accumulation in the seeds and vegetative parts of Camelina widely changed during growth, as observed also by other authors [17]. Rodríguez-Rodríguez et al. reported that the fatty acid distribution in vegetative tissues is related to the plant cells' membrane structures, being different among plant species [17].

Camelina biomass is an interesting source of unsaturated fatty acids, since the leaf tissue is rich in linoleic and α-linolenic acid (18.3 and 4.9% of the total fatty acids, respectively). In accordance with the findings reported by Angelini et al. [48], Camelina produced from the autumn sowing showed good qualitative traits in terms of seed yield and qualitative features as for high concentration of PUFAs. The presence of these two fatty acids is known to occur in the parts of the plant which have high biological activity; Camelina showed high contents of linoleic and α-linolenic acids in leaves, stems and green pods [18].

Camelina undergoes a progressive accumulation of unsaturated fatty acid in seeds [16] and the climatic conditions may influence the fatty acid profile of the different vegetative parts of the plant. High temperatures occurring during flowering and seed ripening interfere with the enzymes responsible of the PUFA metabolism and affect a decrease of their content [16,17], which explains a higher average PUFA concentration (+2%) following the autumn sowing.

4.2. Goat Milk Yield, Chemical and Fatty Acid Composition

Based on a two-year trial, in this study dietary supplementation with Camelina forage harvested at different phenological stages did not influence neither milk yield nor its chemical composition. In the present study, milk yield and chemical composition are comparable to those reported for the Ionica breed in other experiments [49–51].

Goat milk features, especially with regards to the protein and fat content, are known to be influenced by lactation months [52]. In this study, only slight differences were found between sampling periods, and the average protein and fat content in milk from goats fed the two diets are comparable to the values reported by Kováčová et al. [52].

The content of somatic cells in milk from goats fed with Camelina forage was lower as compared to the control one, in particular during the last two sampling periods, corresponding to the month of May. The effect of plant-derived bioactive substances on the inflammatory response of ruminants, including their potential effects on the health of the mammary gland, has been widely documented [53]; whether dietary supplementation with Camelina green biomass may have exerted these beneficial effects has to be ascertained.

The evolution of the presence of somatic cells in milk during lactation is quite controversial; while some authors reported that the SCC remains unchanged throughout the lactation period [52,54], other researchers found a significant increase of the SCC as the stage of lactation advanced [55,56]. The SCC score is used for the evaluation of milk quality, being correlated with the concentration of lactose, udder health and hygiene and dependent on many factors such as breed, month of milking and farming conditions [52–58].

The fatty acid profile of goat milk was markedly affected both by the diet as well as by the milk sampling period. In particular, supplementation with Camelina forage showed a significant increase of the CLA content of milk except for the first stage of stem elongation, with potential benefits for human health since this fatty acid has well-documented health promoting biological properties [59]. The highest concentration of CLA in milk was found when Camelina was harvested during the inflorescence appearance stage (III). Also Pikul et al. [60] found an increase in the concentration of CLA along with that of PUFA in milk from goats supplemented with false flax cake. A markedly lower concentration of SFA was found in the group fed Camelina forage during the last three phenological stages in turn of a greater amount of PUFAs, thus reflecting the accumulation of unsaturated fatty acids occurring in the Camelina plant during growth. As a consequence, the thrombogenicity index of goat milk was always lower in the Camelina group, while the atherogenic index was lower only in milk from goats fed Camelina forage during the fruit set visible stage. In this study, the values found for the atherogenic and thrombogenic indices are in agreement with other results reported for goat milk [52,61]. Previous research has shown that milk from grazing goats has better quality parameters for human nutrition [61,62].

Fresh grass contains a high proportion (50–70%) of fatty acids, mainly linoleic and α-linolenic, and ruminants grazing fresh grass show high levels of n-3 fatty acids in milk and meat [7]. In this study, it may be hypothesized that feeding Camelina green biomass may have amplified the effects of pasture feeding, thus contributing to an increase of the PUFA concentration and to the consequent reduction of the thrombogenic index, in particular during the stage of growth falling between maximum stem elongation and inflorescence appearance.

5. Conclusions

Camelina sativa (L. Crantz) grown in Southern Italy places shows good productive performances in terms of biomass production, confirming its multi-purpose use as oilseed plant, cover and cash cover crop. The crop is feasible, economically sustainable and able to provide seeds, pasture and forage. Camelina harvested during the different phenological stages of growth may be successfully used as fresh forage for autochthonous goats reared under extensive and environmentally friendly systems. Milk obtained from goats fed with Camelina fresh forage has a higher CLA content and lower TI and AI indices, with potential benefits for human health. The combined sustainable agricultural and animal rearing system investigated in this study may contribute to the valorization of typical and traditional products obtained by local autochthonous genotypes that risk genetic erosion such as the Ionica goat breed. Among future research perspectives, it would be interesting to ascertain whether the production of a Camelina silage may be effective for small ruminant feeding, in order to cover the periods of absence of the green biomass and to improve its shelf-life.

Author Contributions: Conceptualization, L.T., F.G. and M.A.C.; methodology, L.T., F.G. and M.A.C.; validation, L.T., M.A.C. and M.S.; formal analysis, L.T.; investigation, L.T., F.G. and M.A.C.; resources, G.D.M.; data curation, L.T. and M.A.C.; writing—original draft preparation, M.A.C.; writing—review and editing, M.A.C., L.T. and M.S.; supervision, G.D.M., V.T. and V.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted within a research project approved by the Ethics Committee for Veterinary Clinical and Zootechnical Studies of the Department of Emergency and Organ Transplantation of the University of Bari (Certificate n. 05/2020).

Acknowledgments: The authors are grateful to Domenico Crocco for his contribution to the field experiment and laboratory assistance.

Conflicts of Interest: The authors declare no conflict of interest.

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