Communication

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Seasonal Variation in Nutritional and Phytochemical Composition of Mediterranean Pasture and Its Impact on Goat Cheese Quality

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Abstract

This study evaluated the seasonal variation (July vs. September) in the chemical and flavonoid composition of Mediterranean natural pasture used for grazing Cilentana goats and assessed its impact on fresh goat cheese quality. A total of 36 pasture samples and 60 cheese samples were analyzed for proximate composition, fiber fractions, minerals, and flavonoids (HPLC). July pastures showed higher dry matter (95.4%), ash (8.74%), and lower fiber (28.7%), whereas September pastures had higher crude protein (17.7%), fiber (43.7%), and kaempferol (36.4 mg/kg DM). Cheese produced in July had higher fat (46.8% DM), ash (8.36%), NaCl (4.78%), and dry matter, while September cheeses showed slightly lower fat (42.2%), higher moisture, and a more acidic pH (5.90). Seasonal increases in kaempferol, lutein, quercetin, and CMG suggest enhanced antioxidant potential in late-season forage. These findings highlight the importance of seasonal pasture monitoring to optimize feed quality and dairy product characteristics in extensive Mediterranean goat systems.

Keywords: flavonoid composition; seasonal variation; cheese quality; Mediterranean pastures; goats

1. Introduction

Flavonoids are a heterogeneous group of secondary metabolites belonging to the polyphenol family, synthesized through the shikimic acid and malonate pathways. They play multiple biological roles, including antioxidant protection and modulation of ecological interactions between plants and herbivores [1,2]. Chemically, flavonoids include several subclasses—flavonones, flavones, flavanones, flavonols, isoflavones, and anthocyanidins—each characterized by distinct structures and activities [3]. Their accumulation in plant tissues is influenced by both genetic factors and environmental conditions such as ultraviolet (UV) radiation, water availability, grazing pressure, and interspecies competition [4,5].

Natural Mediterranean pastures, particularly those in hilly and coastal regions, are highly biodiverse ecosystems that can provide a rich array of bioactive compounds [6,7]. In these extensive grazing systems, flavonoids have relevance on both ecological and zootechnical levels: they act as chemical mediators in plant–plant and plant–animal interactions [8,9], and they can enhance forage quality through their antioxidant and immunomodulatory properties [10]. In small ruminants, the intake of flavonoid-rich forage has been associated with improved ruminal fermentation, reduced methane emissions, and beneficial effects on lipid profiles in milk and meat [11,12]. Nevertheless, most studies have focused on purified extracts or isolated compounds, leaving a gap regarding the natural occurrence and seasonal distribution of flavonoids in spontaneous pastures used in extensive livestock systems [6,7].

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Increasing scientific interest also concerns the transfer of phytochemicals along the food chain, particularly into dairy products. Goat cheese from pasture-raised animals represents an excellent matrix for studying how diet influences the functional and nutritional properties of animal-derived foods [10,13]. Understanding the phytochemical composition of natural forage, and its seasonal variability, is therefore essential for improving feeding strategies, enhancing product quality, and supporting sustainable livestock management.

The present study aimed to characterize the chemical and phytochemical profile of a natural Mediterranean pasture used for goat grazing in southern Italy, quantify seasonal variation of key flavonoids, and evaluate their impact on the chemical composition of goat cheeses produced under extensive grazing conditions.

2. Materials and Methods

2.1. Pasture Analysis

Sampling was conducted in a natural pasture located at sea level in the Sele plain (Salerno, southern Italy), a coastal area bordered by the Picentini Mountains to the north, the Alburni Massif to the east, and the Cilento slopes to the south. The zone is characterized by flat alluvial soils and a typical Mediterranean climate, with average temperatures of 22–28 °C along the coast and 15–20 °C in inland areas, where summers are milder and winters significantly colder [14,15]. The sampling area was centered at approximately 40.50°N, 14.97°E.

The pasture was used for feeding Cilentana goats, a local breed raised under an extensive grazing system. The spontaneous vegetation reflected typical Mediterranean scrub, including Quercus spp., Arbutus unedo, Ceratonia siliqua, Myrtus communis, Pistacia lentiscus, Rosmarinus officinalis, and Juniperus spp., while the herbaceous layer consisted mainly of legumes and grasses. Goats grazed directly on the same areas where samples were collected, and the herd received supplemental feed consisting of 1 kg alfalfa hay and 700 g/head/day corn meal. Pasture sampling was performed manually and randomly along representative transects, cutting vegetation 3 cm below ground level to obtain a composite sample representing the available diet. A total of 36 samples were collected: 18 in July and 18 in September, distributed across six locations.

Samples were transported in refrigerated containers and manually cleaned of impurities. They were then dried in a forced convection oven at 40 °C to constant weight, a temperature selected to minimize thermal degradation of phenolic compounds and preserve the integrity of the flavonoid profile [16,17]. Once dried, samples were finely ground using a stainless-steel blade mill. Chemical analysis followed the methods described in [18] and [19], while net energy values were calculated according to [20]. Ground samples were stored in dark, hermetically sealed glass containers at room temperature, protected from light to prevent oxidation and degradation of bioactive compounds [21].

2.2. Cheese Analysis

Fresh Cilento goat cheese was produced using traditional artisanal methods. Raw milk from the grazing herd was thermized at 85–90 °C for 15–20 min, cooled to 37 °C, and inoculated with liquid goat rennet (0.3 mL/L), allowing coagulation within 60 min. The curd was cut into 1 cm^3 pieces, gently stirred for 15 min, and salted (2% w/w). It was then transferred into cylindrical molds (500 g capacity), pressed at 1.5 bar for 4 h, and ripened for 20 days at 12–14 °C and 85% RH. The resulting semi-soft cheese (DM 71–74%) reflects typical Cilento goat dairy production.

A total of 60 cheese samples were analyzed, with 30 samples derived from each grazing period (July and September). All cheeses originated from the same herd and were produced following identical procedures to ensure consistency.

After production, cheeses were cooled to 4 °C and transported to the laboratory. Chemical composition was assessed according to official analytical methods: AOAC 926.08 for dry matter, method 935.42 for ash, ISO 14156:2001 for lipids, AOAC 920.123 for crude protein, FIL-IDF 17A:1972 for sodium chloride, and ISO 8968-5 for trichloroacetic acid–soluble proteins (TCA 12%) [22]. pH was

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measured in triplicate using a portable pH meter (Crison Basic 25), and mean values were used for statistical analysis. Mean physicochemical parameters (DM, fat, total protein, TCA12, NaCl, pH) are reported in Table 2 for all cheese samples (n = 30 + 30).

2.3. Flavonoid Analysis

Approximately 0.5 g of each dried pasture sample was weighed and placed into 15 mL amber glass tubes to prevent photodegradation of phenolic compounds. Flavonoids were extracted using 80% methanol acidified with 0.1% formic acid, a solvent widely applied for plant matrices due to its ability to solubilize both glycosylated flavonoids and aglycones [23,24]. Acidified methanol has been shown to enhance extraction yield compared to pure methanol or other aqueous systems [25] and is fully compatible with HPLC separation of phenolic compounds [26], while ensuring a comprehensive phytochemical profile [27].

For each sample, 10 mL of solvent were added, and tubes were shaken on a rocking platform at room temperature for 24 h to promote extraction [28]. Samples were then sonicated for 15 min to further improve efficiency, centrifuged at 5000 rpm for 10 min, and the resulting supernatants collected [29]. Extracts were filtered through 0.22 µm PTFE membranes to remove residual particulates [30] and stored at −20 °C until HPLC analysis [31].

Mean flavonoid concentrations and standard deviations are reported in Table 3.

2.4. Statistical Analysis

Descriptive statistics were used for data analysis. Data was analyzed using [32]. The physicochemical composition of the cheese samples and flavonoid content were analyzed using one-way ANOVA (GLM procedure). Statistical significance was set at $p < 0.05$.

3. Results and Discussion

3.1. Pasture Analysis

In July, the pasture showed a DM content of 95.38%, indicating low moisture and a high concentration of nutrients, consistent with typical pasture values of 80–95% DM [33]. Crude protein (15.56%) fell within the optimal range for ruminant forage (10–20%), supporting milk production and growth [34,35]. Ether extract (2.48%) aligned with expected values for grass forages (<5%) [36], contributing modestly to dietary energy. Ash (8.74%) was slightly above the common 4–7% range, indicating elevated mineral content [37]. Total fiber (28.74%) fell within the recommended 25–35% for maintenance diets [38].

Structural fiber fractions (NDF 47.07%; ADF 41.31%) indicated a substantial proportion of structural carbohydrates, potentially reducing digestibility and slowing rumen passage [38,39]. Starch (12.22%) suggested a moderate supply of soluble carbohydrates, supporting rumen function [40]. Energy values (0.62 MFU/kg DM; 0.54 UFV/kg DM) were consistent with medium-quality forage suitable for milk and meat production [34].

Table 1. Mean and STD for physicochemical characteristics of pasture on DM.

Parameter	July (Mean ± STD)	September (Mean ± STD)	p-value
DM (%)	95.38 ± 0.06	94.25 ± 0.29	<0.05*
CP (%)	15.56 ± 0.15	17.71 ± 1.43	<0.05*
Fiber (%)	28.74 ± 0.19	43.67 ± 25.90	<0.01**
Ca (mg/kg)	0.36 ± 0.02	0.80 ± 0.11	<0.01**

In September, DM decreased slightly to 94.25%, reflecting higher moisture likely associated with younger, less mature plant material. CP increased to 17.71%, although the high variability suggests

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differences in species composition or phenological stages across sampling points. EE remained stable (2.47%), while ash declined to 5.54%, likely due to plant senescence or mineral leaching.

Fiber increased markedly to 43.67%, with accompanying rises in NDF (48.12%), ADF (40.13%), and ADL (11.88%), indicating greater lignification and reduced digestibility. Calcium levels doubled (0.80% vs. 0.36%), possibly reflecting late-season physiological changes or soil mineral dynamics. Phosphorus showed a mild decline (0.30%).

Despite the substantial increase in fiber, energy values remained relatively stable (0.62 UFL; 0.50 CFU), likely due to the compensatory effect of higher CP content.

Overall, July pastures presented a more balanced chemical profile, with lower fiber and higher mineral content, while September pastures reflected advanced vegetative maturity, increased fiber, lower ash, and greater compositional variability. These changes align with seasonal plant development and have direct implications for forage digestibility and dairy product quality. The richer energy and mineral profile of July forage corresponds to higher fat and ash levels in July cheeses, whereas the higher fiber and moisture in September forage are reflected in increased acidity and moisture of the corresponding cheeses.

These results emphasize the relevance of monitoring seasonal pasture dynamics to support optimal feeding strategies and maintain consistent cheese quality in extensive grazing systems.

3.2. Cheese Analysis

Cheese produced from milk obtained during July grazing showed a richer nutritional profile, consistent with high-quality forage and favourable early-summer conditions [41,42] (Table 2).

Fat content averaged 46.76%, indicating a high lipid concentration, with variability likely linked to individual animal metabolism, pasture composition, and milk yield. Total protein content (29.95%) aligned with typical values for well-ripened cheeses [41]. TCA-soluble nitrogen (3.93%) indicated moderate proteolysis and the presence of low-molecular-weight peptides [41], suggesting early maturation processes or pasture-related enzymatic influences. Ash content (8.36%) confirmed the elevated mineral availability of July forage, while the NaCl level (4.78%) reflected adequate salting for preservation and flavour development [43].

The elevated dry matter and total solids values indicated a dense, concentrated cheese typical of traditional semi-hard varieties. The pH (6.05) suggested a slightly acidic environment, consistent with intermediate ripening and conducive to proteolytic activity and flavour formation.

Cheese produced from September milk showed compositional modifications reflecting late-season forage conditions. Fat content decreased to 42.17%, consistent with the higher fiber and lignin content of late-maturing pastures, although additional factors related to milk composition and processing may contribute. Despite the lower lipid level, fat remained sufficient to support desirable sensory attributes.

Protein content remained stable between seasons, indicating adequate nitrogen supply from late-season forage. Ash (7.92%) and NaCl (4.27%) showed slight decreases, potentially reflecting reduced mineral availability in senescing plants. Dry matter and total solids were marginally lower, indicating higher moisture content—likely associated with milk composition or curd structure influenced by September forage characteristics. TCA-soluble nitrogen levels were like July values, suggesting comparable proteolytic activity. The pH of September cheese (5.90) was slightly more acidic, possibly due to differences in microbial flora or residual lactose fermentation related to forage biochemical traits.

Overall, cheese composition reflected the seasonal dynamics of pasture. July cheeses benefited from a more favourable nutritional context, with higher fat, mineral content, and dry matter. September cheeses maintained good nutritional value but exhibited traits typical of late-season forage, including higher moisture and increased acidity. These results highlight the importance of managing seasonal variability in grazing systems to ensure consistent dairy product quality.

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Table 2. Mean and STD for physicochemical results for cheeses analyzed on DM.

Parameter	July (Mean ± STD)	September (Mean ± STD)	p-value
Ash (%)	8.36 ± 0.97	7.92 ± 0.83	ns
Fat (%)	46.76 ± 7.81	42.17 ± 6.13	<0.05
NaCl (%)	4.78 ± 0.69	4.27 ± 0.32	<0.01
Protein (%)	29.95 ± 2.46	30.07 ± 1.72	ns
TCA12 (%)	3.93 ± 1.08	3.78 ± 0.78	ns
pH	6.05 ± 0.40	5.90 ± 0.24	<0.05
Total solid (%)	77.59 ± 4.49	76.66 ± 5.76	ns
DM (%)	73.80 ± 4.50	71.30 ± 4.17	ns

3.3. Flavonoid Analysis

In Table 3 the flavonoid content is reported.

Table 3. Mean and standard deviation for flavonoid content.

Parameter	July (Mean ± STD)	September (Mean ± STD)	p-value
Catechin (mg/kg)	0.14 ± 0.01	0.16 ± 0.01	<0.01
Kaempferol (mg/kg)	32.25 ± 1.01	36.36 ± 1.03	<0.01
Daidzein (mg/kg)	0.32 ± 0.01	0.20 ± 0.01	<0.01
Lutein (mg/kg)	0.21 ± 0.01	0.28 ± 0.1	<0.01
Quercetin (mg/kg)	1.13 ± 0.01	1.15 ± 0.01	<0.01
CMG (mg/kg)	1.93 ± 0.01	2.02 ± 0.01	<0.01

The results reported in Table 3 show a clear seasonal modulation of the main phytochemical constituents in the pasture. All measured compounds differed significantly between July and September. Between the two sampling periods, catechin increased by approximately 14%, kaempferol by 13%, lutein by 33%, quercetin by 2%, and CMG by 5%, whereas daidzein decreased sharply by about 38%. These variations indicate an overall late-summer increase in several flavonoids (kaempferol, quercetin) and carotenoids (lutein), accompanied by a marked reduction in the isoflavone daidzein.

These patterns are consistent with previous findings in forage and wild plant species. Monthly variations in kaempferol and quercetin derivatives have been reported in Phillyrea latifolia leaves [44], while long-term studies on multiple tree species have shown seasonal shifts in metabolites derived from both compounds [45]. Carotenoid concentrations, including lutein, also tend to rise during summer, as documented in Brassica vegetables [29]. Flavonoids such as kaempferol and its glycosides typically accumulate under high solar radiation and environmental stress—especially UV exposure—acting as antioxidant and photoprotective compounds in leaves [46]. Similarly, catechin and lutein often increase during late-stage plant maturation as part of metabolic responses to oxidative stress [47].

In contrast, daidzein, an isoflavone commonly found in legumes, generally reaches its highest concentrations in late spring or early summer, then declines during phenological transitions or thermal stress [48,49]. The modest increases in quercetin and CMG suggest tighter metabolic regulation or lower sensitivity to seasonal drivers compared with kaempferol and lutein.

Overall, the pasture's flavonoid profile showed significant seasonal variation ($p < 0.01$ for all compounds). Catechin increased from 0.14 to 0.16 mg/kg, kaempferol from 32.25 to 36.36 mg/kg, lutein from 0.21 to 0.28 mg/kg, and CMG from 1.93 to 2.02 mg/kg, while daidzein decreased from 0.32 to 0.20 mg/kg. These shifts reflect the influence of plant phenology and environmental conditions—light exposure, temperature, and water availability—on the biosynthesis and accumulation of bioactive compounds in grazing species. The late-season rise in several antioxidant flavonoids may represent a plant response to environmental stress or maturation, potentially enhancing the functional properties of forage.

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Such modifications may also influence the nutritional and antioxidant characteristics of animal products. Elevated late-season concentrations of kaempferol and other flavonoids could contribute to the bioactive profile of dairy products, supporting potential benefits for animal health and adding value for consumers seeking functional foods.

Although flavonoid concentrations were relatively low (0.1–36 mg/kg DM), their contribution to the total diet remains relevant, especially considering that grazing increases cheese polyphenol content compared with indoor feeding. Heat treatments such as thermization (85–90 °C) are likely to reduce bioactive compound levels [50]. Future work should include direct HPLC analysis of cheese flavonoids, rumen metabolism trials, and sensory or antioxidant assays to validate the role of pasture phytochemicals in shaping the nutritional characteristics of goat cheese.

4. Conclusions

While statistically significant, flavonoid concentrations (0.1-36 mg/kg DM) were low relative to total diet. Monitoring seasonal forage optimizes cheese quality and sustainability [7,51]. Direct transfer to cheese was not measured, representing a limitation. However, cumulative antioxidant effects from diverse pasture phytochemicals likely contribute to cheese quality.

This study shows clear seasonal differences in the chemical, nutritional, and phytochemical composition of Mediterranean pastures and their impact on goat cheese quality. July pastures had higher dry matter, minerals, and starch, with lower fiber, resulting in cheese with higher fat, ash, and dry matter. September pastures, despite higher protein and certain flavonoids, showed increased fiber and lower mineral content, producing cheese with slightly lower fat, higher moisture, and a more acidic pH. Seasonal increases in kaempferol, lutein, quercetin, and CMG suggest enhanced antioxidant potential in late-season forage. These findings highlight the value of monitoring seasonal changes in forage to optimize feeding, cheese quality, and sustainability in extensive grazing systems.

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