Degree of Antioxidant Protection: A Parameter to Trace the Origin and Quality of Goat's Milk and Cheese

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ABSTRACT

Traceability is an essential tool in reassuring consumers and traders that food is as safe, authentic, and of good quality as expected. Today, food traceability procedures often consist of attached documents and declarations, but scientific parameters that could objectively identify a product would be preferable. Scientific efforts in this area are mostly focused on selection and validation of experimental indicators that would be useful for tracing a food product in any step of its commercial life, describing its raw materials, processing procedures, and storage conditions. In this research, milk and cheese samples from zero grazing and grazing goats were studied to identify a tracing parameter correlated to the feeding system. In particular, α -tocopherol and cholesterol were analyzed by HPLC on a normal phase column and were combined to calculate the degree of antioxidant protection (DAP). This parameter, expressed as the molar ratio between antioxidant compounds and an oxidation target, is useful for tracing and distinguishing products from grazing and zero-grazing animals. Degree of antioxidant protection values greater than 7.0×10^{-3} were found in samples from grazing goats and values lower than 7.0×10^{-3} were found in samples from zero-grazing goats, for both milk and cheese, meaning that cholesterol was highly protected against oxidative reactions when herbage was the only feed or was dominant in the goat diet. The reliability of DAP to measure the antioxidant protection of cholesterol appeared more effective when the feeding system was based on grazing than when cut herbage was utilized indoors by animals. The DAP index was able to distinguish dairy products when the grazed herbage in the goats' diet exceeded 15%.

Key words: tracing parameter, antioxidant protection, goat milk, cheese

INTRODUCTION

In recent years, the need for high-quality, safe, and nutritious foods has been increasing. To meet consumer demand, the dairy industry has developed and optimized ad hoc technologies that improve food natural quality by adding compounds such as essential fatty acid n-3, vitamins, minerals, probiotics, antioxidants, and other ingredients. Such fortified foods are not the result of natural animal production, but are the expression of industrial technology. On the other hand, consumers demand "authentic" and natural products, not products artificially enhanced with functional compounds. Consequently, food traceability is becoming more important worldwide as a tool to follow products from the point of origin to the final step. Product traceability determines the physical location of a product at any stage in the supply chain to facilitate logistics and inventory management, product recall, and dissemination of information to consumers (Opara, 2003).

Data concerning traceability are important for consumers and should be as complete as possible. In particular, elements concerning the role of the feeding system to determine the functional quality of milk and dairy products are often missing or incomplete.

The main objective of this article was to identify and propose a parameter as a tracing tool to identify milk and cheese from different feeding systems (grazing and zero grazing). This tracing parameter, the degree of antioxidant protection (**DAP**), calculated on the basis of α -tocopherol and cholesterol contents, allows an evaluation of milk and cheese resistance to oxidative reactions, the main determinants of food quality and functionality for human nutrition.

MATERIALS AND METHODS

Location and Animal Management

The experiments were carried out at Bella (40°21' N; 15°30'25" E), 360 m above sea level, and Li Foy (40°37' N; 15°42' E), 1,200 m above sea-level, the 2 experimental farms of the Istituto Sperimentale per la Zootecnia (CRA) located in the Basilicata region of southern Italy.

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Table 1. Experimental design

Experiment	Group ¹	n	Forage	Concentrate, ² kg/d	Product
Concentrate grain sources (experiment 1)	G	15	Grazing 8 h/d	0	Milk
	GBC	15	Grazing 8 h/d	0.6 BC (73 + 27)	Milk
	GMB	15	Grazing 8 h/d	0.6 MB (78 + 22)	Milk
	ZG	15	Hay ad libitum	0.6 CC	Milk
Concentrate ad libitum (experiment 2)	G	15	Grazing 8 h/d	0	Cheese
	GU	15	Grazing 8 h/d	Mix, ad libitum ³	Cheese
	ZGU	15	Hay ad libitum	Mix, ad libitum ⁴	Cheese
Grazing environment (experiment 3)	GV	16	Grazing 8 h/d	0	Cheese
	GM	16	Grazing 8 h/d	0	Cheese
	ZG	16	Hay ad libitum	0.6 CC	Cheese
			Herbage, g of DM/d	To complete the diet	
Herbage grazed (experiment 4)		15	0	CC	Milk
		15	450	CC	Milk
		15	600	CC	Milk
		15	1,100	CC	Milk

¹G = grazing, GBC = grazing + mixed barley and chickpeas, GMB = grazing + mixed corn and broad beans, ZG = zero grazing, GU = grazing + concentrate ad libitum, ZGU = pasture hay and concentrate ad libitum, GV = grazing on valley pasture, GM = grazing on mountain pasture.

²BC = barley + chickpeas; MB = corn + broad beans; CC = commercial concentrate; mix = 45% barley + 25% chickpeas + 20% beet-pulps + 10% broad beans.

³Real intake: 1.2 kg/d.

⁴Real intake: 1.3 kg/d.

The animals used for these experiments were selected from the 2 herds of CRA-Istituto Sperimentale per la Zootecnia, Potenza. For each farm, 6 ha of native herbageous pasture were divided into 6 equal paddocks, alternately grazed by the experimental groups of goats.

Feeding Treatments and Sampling

Four experiments were carried out to evaluate the effects of 1) different concentrate grain source supplementations on milk quality, 2) ad libitum concentrate supplementation on cheese quality, 3) different grazing environments on cheese quality, and 4) grazed herbage on milk quality.

Experiment 1: Effect of Grain Source Supplementation. Mature Maltese goats were blocked into 4 homogeneous groups by BCS and milk yield ($1,460 \pm 62$ mL/d) and assigned to 1 of 4 feeding treatments (Table 1): grazing 8 h/d, no concentrate (G); grazing + mixed barley and chickpeas (GBC); grazing + mixed corn and broad beans (GMB); and zero grazing (ZG). In each concentrate, CP and NDF contents were similar (150 and 180 g/kg of dry material respectively). Milk collection started in March after 2 mo of grazing (native pasture) at 100 ± 12 d of lactation. Milk was collected also in April, May, June, and July (1 sample per treatment per head per month). This experiment was repeated in 2 subsequent years.

Experiment 2: Effect of Ad Libitum Supplementation. Maltese goats were blocked into 3 homogeneous groups by BCS and milk yield ($1,173 \pm 48$ mL/d) and

assigned to 1 of 3 feeding treatments: unsupplemented grazing goats (GC); grazing plus unlimited concentrate (GUC); and zero grazing plus unlimited concentrate (ZGUC). This experiment was carried out for 1 yr. From spring to summer, milk was collected, filtered, and processed into Caciotta cheese, an artisanal goat cheese typical in southern Italy. Three cheeses were produced by heating raw milk at 36°C and adding liquid calf rennet at 35 mL/100 L. The curd formed in approximately 20 to 25 min and was cut into 10 cm × 10 cm blocks. After 5 min of rest, the curd was placed into cylindrical molds of 113 × 80 mm. After 24 h the cheese was stored in a natural cave and ripened for 5 mo at 8 to 10°C and 80 to 85% relative humidity.

Experiment 3: Effect of Grazing Environments. Maltese goats were blocked into 3 homogeneous groups by BCS and milk yield ($1,165 \pm 65$ mL/d) and assigned to 1 of 3 feeding treatments (Table 1): grazing on valley pasture (GVC); grazing on mountain pasture (GMC); and zero grazing (ZGC). This experiment was carried out for 1 yr. Cheese preparation was carried out as described in the previous experiment.

Experiment 4: Effect of Grazed Herbage. Grazing Maltese goats were blocked into homogeneous groups and assigned to different grazed herbage amount treatments (Table 1): 0, 450, 600, or 1,000 g of DM/d with commercial concentrate to complete the diet. To complete the diet, suitable amounts of a concentrate based on corn, barley, beet pulp, chickpeas, and broad beans were utilized. In this experiment, milk was sampled in

the last 3 d of a 2-wk period from lactating goats after 1 mo of grazing adaptation.

Samples

Weighed milk aliquots were immediately sampled in disposable tubes and stored at -20°C . Milk samples were analyzed using the entire contents of each tube.

The Caciotta cheeses were sampled after ripening, according to the FIL-IDF sampling procedures (FIL-IDF, 1980), cleaned of inedible parts, and cut into small pieces. After chilling to reduce losses of fat and moisture, small pieces were grated using a Waring blender and combined to create homogeneous representative samples.

Analytical Methods

α -Tocopherol and cholesterol were determined by the following method: all samples were hydrolyzed in alkaline solution and the extracted residue was dissolved in 2-propanol (1%) in *n*-hexane and analyzed by the normal phase chromatographic method described in Panfili et al. (1994).

Chromatography was performed using an HPLC analytical system comprising a Waters Model 510 solvent delivery system and a Gilson autosampling 231-401 injector.

The normal phase column was a $250 \times 4.6 \text{ mm} \times 5\text{-}\mu\text{m}$ Beckman Ultrasphere Si (Beckman Coulter, Fullerton, CA). A back pressure regulator containing a replaceable cartridge (Upchurch Scientific Inc., Oak Harbor, WA) enables the pump to operate more efficiently, warranting a 3.45-MPa pressure increase during the normal phase chromatographic run. Chromatographic solvents were 2-propanol (1%) in *n*-hexane (A) and *n*-hexane (B) in a multilinear gradient elution at a flow rate of 1.5 mL/min (Panfili et al., 1994).

A programmable multiwavelength spectrophotometer (model 490, Waters, Milford, MA) and a programmable spectrofluorometer (model L540, Perkin Elmer, Waltham, MA) were connected in series to determine, in the same chromatographic run, tocopherols (excitation 280 nm, emission 325 nm) and cholesterol (210 nm).

Each analysis was done in triplicate and results were calculated by a Millennium chromatography system (Waters). Standards of α -tocopherol and cholesterol were obtained from Sigma Chemical Co. (Milan, Italy).

DAP Calculation

The DAP, proposed as the tracing parameter, was calculated as the molar ratio between antioxidant compounds and a selected oxidation target:

$$DAP = \frac{\sum_{i=1}^n AC_i (n^{\circ}\text{moles})}{OT (n^{\circ}\text{moles})}$$

where *AC* is the antioxidant compound (α -tocopherol), *OT* is the oxidation target (cholesterol) and *i* is the number of components.

To facilitate reading and comparison among samples, the DAP number is expressed in an exponential form ($\times 10^{-3}$).

This index can be used to evaluate the antioxidant protection of food products, selecting the proper antioxidants and oxidation target. For instance, α -tocopherol, β -carotene, and polyphenols represent a natural and particularly efficient antioxidant system in extra virgin olive oil, whereas linoleic acid, an oxidizable unsaturated fatty acid, can be selected as the oxidation target. Their combination in the DAP parameter is useful as a predictive index of oil sensitivity to oxidation during storage (Esti et al., 2004).

In dairy products from goats, only α -tocopherol was selected as the antioxidant because of the absence of detectable levels of β -carotene in goat's milk, and cholesterol was the oxidation target because of the low content of oxidizable unsaturated fatty acids in milk fat (Pizzoferrato and Manzi, 1999; Pizzoferrato et al., 2000). Moreover, even if less easily oxidizable than unsaturated fatty acids, cholesterol is a molecule usually charged, especially in the oxidized form; cholesterol oxides are responsible for heart disease in humans (Caponi et al., 1994; Brown and Jessup, 1999).

The effectiveness of the DAP parameter to distinguish milk and cheese from grazing and zero-grazing animals was evaluated in experiments 1, 2, and 3; DAP's relationship with actual herbage intake was evaluated in experiment 4.

Statistical Analysis

The results of milk and cheese chemical composition were submitted to ANOVA. Statistical treatment was performed using the KaleidaGraph 3.6 software (Synergy Software, Reading, PA). When the results of the ANOVA were significant ($P < 0.05$), means were compared using Tukey's significant difference test. In consideration of the large variability shown by animals, only values of $P > 0.10$ were considered to be nonsignificant.

Data from the experiment 4 were submitted to a linear fitting procedure to identify the mathematical equation correlating herbage intake and DAP values.

Table 2. The effect of different concentrate grain sources on milk α -tocopherol, milk cholesterol content, and DAP values¹

Feeding treatment ²	α -Tocopherol, $\mu\text{g}/100\text{ g}$	Cholesterol, $\text{mg}/100\text{ g}$	DAP, $\times 10^{-3}$
First year			
GM	154.9 $\pm$ 76.7 ^a	14.7 $\pm$ 4.3	9.1 $\pm$ 1.7 ^a
GBCM	118.9 $\pm$ 38.4 ^{ab}	11.8 $\pm$ 2.1	8.9 $\pm$ 1.4 ^{ab}
GMBM	125.2 $\pm$ 31.2 ^{ab}	12.8 $\pm$ 2.8	8.7 $\pm$ 0.7 ^a
ZGM	77.1 $\pm$ 8.4 ^b	12.8 $\pm$ 1.1	5.4 $\pm$ 0.4 ^b
Second year			
GM	169.5 $\pm$ 67.6 ^a	13.4 $\pm$ 1.2	11.3 $\pm$ 0.4 ^a
GBCM	131.6 $\pm$ 53.5 ^{ab}	11.8 $\pm$ 1.3	9.8 $\pm$ 0.3 ^{ab}
GMBM	105.4 $\pm$ 2.8 ^{ab}	11.0 $\pm$ 2.3	8.8 $\pm$ 1.6 ^{ab}
ZGM	67.0 $\pm$ 6.9 ^b	12.1 $\pm$ 1.0	5.0 $\pm$ 0.4 ^b
First and second year			
GM	161.4 $\pm$ 68.7 ^a	14.1 $\pm$ 3.2	10.1 $\pm$ 2.9 ^a
GBCM	124.6 $\pm$ 43.1 ^a	11.8 $\pm$ 1.7	9.3 $\pm$ 2.1 ^a
GMBM	119.6 $\pm$ 27.3 ^{ab}	12.3 $\pm$ 2.6	8.7 $\pm$ 0.9 ^a
ZGM	72.6 $\pm$ 9.0 ^b	12.5 $\pm$ 1.0	5.2 $\pm$ 0.4 ^b

^{a,b}Means within each column within a year with different superscripts differ ($P < 0.05$).

¹Mean data from 5 samples/yr collected in March, April, May, June, and July and analyzed in triplicate $\pm$ standard deviation.

²Feeding treatments: GM = not supplemented grazing goats; GBCM = grazing plus 0.6 kg/d of mixed barley and chickpeas grain; GMBM = grazing plus 0.6 kg/d of mixed corn and broad beans grain; ZGM = pasture hay ad libitum plus 0.6/kg/d of commercial concentrate.

RESULTS AND DISCUSSION

Experiment 1: Effect of Grain Source Supplementation

α -Tocopherol and cholesterol contents in milk samples are reported in Table 2. No significant differences in cholesterol contents were observed among groups, whereas α -tocopherol content shows the lowest value in ZGM (milk from ZG treatment) treatment and the highest in the GM treatment ($P < 0.10$ and $P < 0.05$ for the first and second year, respectively, and $P < 0.01$ for the merged data). The DAP values are also reported in Table 2; DAP showed a decreasing trend in milk: GM > GBCM > GMBM > ZGM. The most significant differences were observed in milk from GM and ZGM treatments: $P < 0.05$ for each single year and $P < 0.01$ for the merged data. The ZGM data showed the lowest values compared with GBCM ($P < 0.10$) and GMBM ($P < 0.05$) treatments.

According to these results, the DAP index is able to differentiate products from grazing and zero-grazing treatments in spite of the high seasonal variability of α -tocopherol in milk from grazing animals. α -Tocopherol in milk reached the minimum value in April and May (95.3; 88.0 and 94.1 $\mu\text{g}/100\text{ g}$ in the GM, GBCM, and GMBM treatments, respectively) and the maximum in July (279.4; 197.6 and 174.8 $\mu\text{g}/100\text{ g}$ in the GM, GBCM, and GMBM treatments, respectively).

Table 3. The effect of concentrate supplementation level on cheese α -tocopherol and cholesterol content and DAP values¹

Feeding treatment ²	α -Tocopherol, $\mu\text{g}/100\text{ g}$	Cholesterol, $\text{mg}/100\text{ g}$	DAP, $\times 10^{-3}$
GC	775.2 $\pm$ 59.1 ^a	92.3 $\pm$ 8.2	7.6 $\pm$ 0.5 ^a
GUC	553.0 $\pm$ 58.2 ^c	100.4 $\pm$ 5.0	4.9 $\pm$ 0.4 ^c
ZGUC	423.0 $\pm$ 29.9 ^b	99.5 $\pm$ 3.4	3.8 $\pm$ 0.3 ^b

^{a-c}Means within each column with different superscripts differ ($P < 0.05$).

¹Mean data from 3 samples collected during lactation and analyzed in triplicate $\pm$ standard deviation.

²Feeding treatments: GC = unsupplemented grazing goats; GUC = grazing plus unlimited concentrate; ZGUC = zero grazing plus unlimited concentrate.

Considering that the highest herbage intake was observed in April and May and not in July (30 to 40%), it was supposed that the observed variability was due to different α -tocopherol content in the grazed herbage (Wilmonth et al., 2000). In the same experiment, α -tocopherol content ranged from 57.1 to 81.0 $\mu\text{g}/100\text{ g}$ in ZGM from April to July, with a narrower range of variability and a lower amount than in the grazing milk.

On the whole, these results show that cholesterol and α -tocopherol contents singularly do not represent effective parameters to differentiate grazing or zero-grazing products even if greater contents of α -tocopherol are always observed in milk from grazing animals. The DAP values seemed to be more useful for evaluating the effects of feeding treatment on product quality ($P < 0.001$) and for differentiating, at least, milk from zero-grazing and grazing animals. These data are confirmed in Table 3 and Table 4 in which the results of the second and the third experiments are shown.

Experiment 2: Effect of Ad Libitum Supplementation

In the second experiment (Table 3), no significant differences in cheese cholesterol content were observed

Table 4. The effect of 2 different pastures on cheese α -tocopherol and cholesterol content and DAP values¹

Feeding treatment ²	α -Tocopherol, $\mu\text{g}/100\text{ g}$	Cholesterol, $\text{mg}/100\text{ g}$	DAP, $\times 10^{-3}$
GVC	778.3 $\pm$ 57.6 ^a	84.5 $\pm$ 4.9 ^a	8.3 $\pm$ 0.7 ^a
GMC	842.0 $\pm$ 36.5 ^a	87.2 $\pm$ 0.7 ^a	8.7 $\pm$ 0.3 ^a
ZGC	646.9 $\pm$ 72.7 ^b	96.7 $\pm$ 6.1 ^b	6.0 $\pm$ 1.0 ^b

^{a,b}Means within each column with different superscripts differ ($P < 0.05$).

¹Mean data from 3 samples collected during lactation and analyzed in triplicate $\pm$ standard deviation.

²Feeding treatments: GVC = grazing on valley pasture; GMC = grazing on mountain pasture; ZGC = zero grazing.

($P > 0.10$), whereas α -tocopherol showed significantly ($P < 0.001$) greater levels in cheese from grazing treatments (GC and GUC) than from the zero-grazing treatment ($P < 0.001$). No significant differences were observed between the 2 grazing groups, despite the fact that GUC was supplemented with an unlimited amount of concentrate; this confirmed the ineffectiveness of α -tocopherol alone as a feeding indicator.

Experiment 3: Effect of Grazing Environment

Cheese samples from the third experiment (Table 4) showed levels of α -tocopherol significantly lower in the zero-grazing treatment (ZGC) than in the mountain pasture treatment (GMC; $P < 0.05$), whereas less significant differences ($P < 0.10$) were observed compared with the valley pasture treatment (GVC). Only in this experiment was an effect of feeding treatment on cholesterol content observed: cholesterol concentration was higher in the ZGC treatment than in the GVC ($P < 0.05$) and GMC ($P < 0.10$) treatments.

The effectiveness of the DAP index was confirmed by these last 2 experiments. The DAP value was high when pasture herbage was dominant in the feeding treatment, and the differences were significant for experiment 2 ($P < 0.001$) and experiment 3 ($P < 0.05$; Table 3 and Table 4, respectively).

The results of the 3 experiments showed that the products from grazed goats were richest in α -tocopherol and contained a cholesterol molecule highly protected (i.e., high DAP values) against oxidative reactions. Cholesterol protection was improved when animals were fed exclusively on herbage pasture or when this feed was dominant in the animal diet.

This statement can be visualized by plotting the DAP value as a function of cholesterol concentration in each sample analyzed (Figure 1). It is interesting to note that, within each group of products (milk and cheese), higher DAP values were found in the samples from grazing goats (open symbols) than in the zero-grazing goats (solid symbols) and the borderline, for both milk and cheese samples, was located at the same DAP value (7.0×10^{-3}).

Nevertheless, in the zero-grazing (ZGC, ZGM, ZGUC treatments) section of the plot, below the threshold value, cheese samples from ad libitum-supplemented grazing goats (GUC) could be observed. This means that the DAP index has a limit of detectability in its capacity to distinguish products from different grazing systems. To better understand this aspect, the fourth experiment was carried out to study the effect of different amounts of grazed herbage in grazing goats, compared with a zero-grazing group.

Figure 1. Relationship between degree of antioxidant protection (DAP) and cholesterol content in milk and cheese from different feeding treatments: GM and GC = not supplemented grazing goats; GBCM = grazing plus mixed barley and chickpeas; GMBM = grazing plus mixed corn and broad beans; ZGM = pasture hay ad libitum plus 0.6 kg/d of commercial concentrate; GVC = grazing on valley pasture; GMC = grazing on mountain pasture; ZGC = zero grazing; ZGUC = zero grazing plus unlimited concentrate; GUC = grazing plus unlimited amount of concentrate. Treatment names ending in M indicate analysis in milk; treatment names ending in C indicate analysis in cheese.

Experiment 4: Effect of Grazed Herbage

Herbage intake was estimated on the basis of herbage mass, measured on 2×2 m of pasture before and after grazing. The real intake of grazed herbage for each group was 0 (control fed on commercial concentrate), 450, 600, and 1,100 g of DM/d, and the contribution of grazing to the diet in each treatment was calculated as a percentage of the maximum actual herbage intake [from 0 g/d (0% grazing) to 1,100 g/d (100% grazing)]. Cholesterol and α -tocopherol contents of milk were determined and, on this basis, the DAP values were calculated. The relevant results are reported in Figure 2 as a function of grazing (%).

The linear equation ($y = a + bx$) fit the experimental points with a high correlation coefficient ($R^2 = 0.97$) and can be utilized as an analytical calibration curve.

Replacing the variable y with the DAP threshold value, able to differentiate “pasture” products from “stabled” ones ($\text{DAP} = 7.0 \times 10^{-3}$), the x value can be calculated. This value (15%) can be considered the limit of detection of DAP.

If the same amounts of freshly cut pasture herbage were ingested indoors and not grazed by animals, the linear fitting of DAP values vs. the amount of cut herbage intake would show a lower correlation value ($R^2 =$

Figure 2. Relationship between milk degree of antioxidant protection (DAP) and grazed herbage intake. Each point is the mean of triplicate analyses of milk collected from homogeneous groups of goats grazing herbage. The x-axis indicates the contribution of grazed herbage intake to the animal diet calculated as a percentage of the maximum intake (1,100 g/d = 100% grazing).

0.62, data not shown). Probably the herbage intake was not the main determinant of the DAP value. Grazing allows goats to select their favorite herbage and plays a key role in improving animal well-being and milk composition.

CONCLUSIONS

In this research we successfully developed the DAP parameter, which was used to differentiate products from grazing and zero-grazing feeding systems, provided that grazed herbage exceeded 15% in the animals' total diet. In fact, grazing action, not just the intake of herbage cut from pasture, is a determinant of milk and

cheese commercial quality, contributing to price setting and probably to animal well-being. Moreover, the DAP parameter was found to be related to oxidative reactions, the main determinant of quality loss in food: the higher the DAP value, the greater the product stability and safety, considering the risk related to the intake of cholesterol-oxides in humans.

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