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The inclusion of fresh forage in the lactating buffalo diet affects fatty acid and sensory profile of mozzarella cheese

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ABSTRACT

The aim of this study was to determine the effect of inclusion of fresh forage in diet for lactating buffalo on properties of mozzarella cheese under intensive farming conditions. Thirty-two buffalo cows were equally allotted into 2 groups fed diets with (fresh group, FRS) or without (control group, CTL) fresh sorghum. The study consisted of 2 trials. In the first one, animals from group FRS were fed a diet containing 10 kg of fresh sorghum (10-FRS diet) that was doubled to 20 kg (20-FRS diet) in the second trial. All diets were isonitrogenous and isoenergetic, and fresh forage accounted for 13.4 and 26.5 of dietary dry matter, respectively, for the 10-FRS and 20-FRS diet. In each trial, milk from the 2 groups was used to produce 3 batches/diet of Mozzarella di Bufala Campana Protected Designation of Origin cheese. Milk yield and composition were not influenced by dietary treatment. The use of 10-FRS diet did not affect any properties of mozzarella. As the inclusion rate of fresh sorghum doubled to 20 kg, an increment of unsaturated fatty acid percentages and a lowering of short-chain and saturated fatty acids were observed. Moreover, the sensory characteristics of mozzarella were modified, although no effects were observed on consumer acceptance. We conclude that the use of green fodder can represent a low-cost feeding strategy to improve the healthiness of buffalo mozzarella under intensive farming conditions with no detrimental effect on consumer blind acceptance.
Key words: fresh forage, buffalo mozzarella cheese, fatty acid composition, sensory properties

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INTRODUCTION

Dairy water buffalo (*Bubalus bubalis*) farming is a traditional Italian enterprise that in recent years has been involved in intensification of rearing techniques (Napolitano et al., 2004). Buffalo milk is almost exclusively used for cheese-making mozzarella (Masucci et al., 2016), a typical fresh and stringy-textured cheese, that has been endowed (EC 103/2008) with Protected Designation of Origin (**PDO**) Mozzarella di Bufala Campana. In the last few years, an increasingly number of buffalo farms have spread outside the PDO area to take advantage of the high price paid for buffalo milk and to differentiate dairy products (Cecchinato et al., 2012). In such increasingly competitive market, buffalo dairy farmers producing mozzarella-PDO are forced to pursue competitive strategies focusing on product quality. The increasing consumer interest in nutritional and health properties of foods could create new market opportunities (Jones and Jew, 2007; Siró et al., 2008; Annunziata and Vecchio, 2011).

Dietary recommendations for human health indicate a reduction of SFA and *trans* fatty acids to reduce incidence of cardiovascular disease (Kliem and Shingfield, 2016). Depending on breed, diet, and stage of lactation, fat of milk and dairy products has SFA content over 60%, but it contains the health-promoting rumenic acid (*cis*-9,*trans*-11 C18:2, commonly referred as CLA), a naturally occurring anticarcinogen (Jensen, 2002). Therefore, interest is growing in the development of dairy products naturally enriched in PUFA and CLA. Several feeding strategies are known to be able to provide higher nutritional characteristics to milk fat (Chilliard and Ferlay, 2004; Elgersma et al., 2006). In particular, the use of fresh forage may represent a low-cost approach in comparison with diet supplementation with oilseeds or fats and does not result in significant increases in *trans* 18:1 isomers other than *trans*-11 18:1 (Dewhurst et al., 2006). In addition, consumers commonly prefer "green image" products obtained from grazing animals or, at least, fed without the use of preserved fodders (Kalač, 2011).

However, feeding management and fat characteristics may also affect the sensory quality of the dairy products (Coulon et al., 2004; Dewhurst et al., 2006), whereas any perceived reduction of typical characteristics of a traditional food might be not accepted by regular consumers (Vecchio et al., 2016).

Total mixed rations based on maize and grass silages, hays, and concentrates are commonly used in buffalo farming throughout the year. The hypothesis is that fresh-cut forage inclusion in the diet for lactating buffalo would be able to improve the healthy characteristics of milk fat under intensive farming conditions. We used sorghum, a forage crop that is spreading in intensive dairy farming, due to its higher flexibility (compared with maize silage, it can be used both fresh and ensiled), and lower environmental impact (compared with maize silage it needs lower inputs of water and nitrogen fertilizer; Lemaire et al., 1996; Farré and Faci, 2006). Therefore, this study aimed to evaluate fatty acid profile, sensory properties, color, and consumer liking of Mozzarella di Bufala Campana PDO cheese produced under the dietary use of fresh sorghum.

MATERIALS AND METHODS

Experimental Design, Animals, Diets, and Cheese Production

The study consists of 2 trials, 17 d each, and took place in September 2014 in a buffalo dairy farm (40°31′N 14°57′E, Campania region, southern Italy) producing PDO mozzarella cheese. Thirty-two lactating buffalo were blocked by milk yield and DIM and randomly allocated into 2 groups fed diets with (fresh group, **FRS**) or without (control group, **CTL**) daily cut fresh sorghum. Two inclusion rates of fresh sorghum (10 and 20 kg as fed) were tested. The lower rate was chosen in order not to markedly change the daily feeding routine and also to extend the period of fresh forage availability. However, this lower rate was unable to significantly change mozzarella fatty acid profile; therefore, it was doubled. The cows were housed into 2 adjacent freestall barns with access to the outdoors and were milked twice daily (0500 and 1700 h).

In the first trial, the CTL group was fed the standard diet used by the farmer, whereas the FRS group was fed a TMR containing 10 kg of fresh sorghum (**10-FRS** diet). Fresh forage accounted for about 13.4% of TMR on a DM basis. The CTL and 10-FRS diets were formulated to be isonitrogenous and isoenergetic and were based on the same ingredients except for inclusion of fresh forage (Table 1). Sorghum [*Sorghum bicolor* (L.) Moench × *Sorghum sudanense* (Piper) Stapf.; commercial hybrid Nicol, Pioneer Hi-Bred International, Johnston, IA] was sown on the farm in July 2014 and at start of the trial was at the early milk stage [i.e., growth stage 5 to 6 according to the scale of Vanderlip and Reeves (1972)]. Sorghum was cut daily about 3 cm long and was mixed into the TMR with the other ingredients. The rations were fed once daily (0800 h) for ad libitum intake (approximately 10% orts) and were re-approached twice daily to ensure unlimited access to feed. The experimental period consist of 14 d of adaptation to diet and 3 d of cheese manufacturing. In each of them, daily (sum of pm and am milkings) bulk milk of each group was collected along with sampling of fresh sorghum, TMR, and milk of each cow. Group milk was transported to the dairy in refrigerated stainless-steel tanks and used for separately manufacturing mozzarella cheese according to the traditional procedure. Briefly, raw water buffalo milk was gently heated (37–38°C) and added with natural whey starter culture from the previous day manufacture and liquid rennet (Caglificio Clerici S.p.a., Codrago, Como, Italy). At curd formation, the coagulum was reduced to particles of 2 to 3 cm and held under whey until pH 4.85, a value suitable for manual stretching into hot water (90–95°C). Thereafter, the stretched curd was mechanically formed into 50-g small balls that were placed in brine (2% NaCl) and sent to the laboratory. A total of 3 batches/diet were produced, about 20 kg each. Over the 3 d of cheese manufacturing, yield (%) of mozzarella was 27.2 ± 0.21 and 27.1 ± 0.26, and DMI (kg/d) was 18.2 ± 1.15 and 17.9 ± 0.65 for CTL and 10-FRS groups, respectively.

The second trial started immediately after the end of the first one. Other 32 animals were used and randomly allocated to the CTL and FRS-20 groups. The TMR for control group was kept constant, whereas group FRS was fed a diet in which the fresh sorghum content was doubled to 20 kg (**20-FRS** diet) and accounted for about 26.5% of DM. The 20-FRS diet was kept isonitrogenous and isoenergetic with respect to CTL (Table 1). Fresh sorghum was at the soft dough stage [i.e., growth stage 7 (Vanderlip and Reeves, 1972)]. The same experimental design and sampling procedure reported above for the first trial were used. Average percent mozzarella yields were 28.6 ± 0.59 and 28.6 ± 0.80, whereas DMI (kg/d) were 19.1 ± 0.8 and 18.9 ± 0.9 for groups CTL and 20-FRS, respectively.

Chemical Analyses of Milk, Feeds, and Cheese, and Instrumental Measures of Color and Texture of Cheese

The samples of fresh sorghum and TMR collected over the 3 d of mozzarella manufacturing were pooled

Table 1. Ingredients and chemical composition of fresh sorghum (n = 2) and of the TMR fed to buffalo cows

Item	Fresh sorghum	CTL TMR[1]	10-FRS TMR[2]	20-FRS TMR[3]
Ingredient, kg as fed				
Fresh-cut sorghum	—	—	10	20
Maize silage	—	18	18	15
Grass silage	—	10	7.0	4.0
Meadow hay	—	4.5	3.0	2.5
Maize meal	—	1.5	2.0	3.2
Ground corn silage	—	2.5	1.5	—
Concentrate[4]	—	2.8	3.3	3.1
Mineral and vitamin mix	—	0.25	0.25	0.25
DM, kg	—	17.6	18.0	18.2
Forage % DM	—	68	69	69
Chemical composition				
DM, %	23.7	—	—	—
NE_L, MJ/kg of DM	4.76	5.94	6.00	5.95
Ether extract, % of DM	2.5	2.8	2.9	3.0
CP, % of DM	7.1	14.5	13.6	13.7
NDF, % of DM	45.7	42.2	39.5	38.8
ADF, % of DM	34.9	28.0	28.5	29.1
Starch, % of DM	2.91	20.2	19.8	18.1

[1]Total mixed ration containing no fresh sorghum.

[2]Total mixed ration containing 10 kg of fresh sorghum.

[3]Total mixed ration containing 20 kg of fresh sorghum.

[4]Based on soybean meal, sunflower meal, and barley meal.

by type and analyzed for DM (air-dried oven at 65°C until constant weight), CP (Kjeldahl method), ash and ether extract (AOAC International, 2002), and NDF and ADF (Van Soest et al., 1991). The NE_L of the diets was calculated according to Sauvant and Nozière (2013).

Milk samples were analyzed the same day of collection for fat, protein, and lactose (MilkoScan FT 6000, Foss Electric, Hillerød, Denmark). Each batch of mozzarella (6 for CTL, 3 for 10-FRS diet, and 3 for 20-FRS diet) was separately analyzed the day after manufacturing. Overnight, the samples were stored at 10°C and were allowed to equilibrate at room temperature (22–23°C) before analysis. The color, chemical composition, and fatty acid composition were determined on 3 samples/batch about 200 g each. For each sample, the color was determined in quadruplicate and was separately measured in the inner and outer surface of cheese according the CIELAB system (spectrophotometer U-3000, Hitachi, Tokyo, Japan). Chemical and fatty acid compositions of each sample were determined in triplicate after grinding the samples. Moisture was determined by oven drying; fat and protein were quantified by the Gerber and Kjeldahl methods, respectively (AOAC International, 2002). Extraction of fat for fatty acid composition was carried out according the Schmidt–Bondzynski–Ratzlaff method with modifications as described by Romano et al. (2011). The GC analysis was performed on a DANI Master gas chromatograph (Dani Instrument SPA, Cologno Monzese, Milan, Italy) instrument equipped with a Quadrex Bonded Cyanopropyl silicone capillary column (length 60 m, internal diameter 0.25 mm, film thickness 0.25 μm) according to the procedure outlined elsewhere (Esposito et al., 2014). Fatty acid peaks in chromatograms were identified using the Supelco 37 Component FAME MIX (Supelco, Bellefonte, PA). Standards for CLA (C18:2 *cis*-9,*trans*-11) and *trans* vaccenic acid (C18:1 *trans*-11) were obtained from NuChek Prep (Elysian, MN). Values of individual fatty acids <0.1 were not quantified. Fatty acids were expressed as a percentage of total methylated fatty acids. Atherogenic index was calculated according to Ulbricht and Southgate (1991).

Sensory Analyses of Mozzarella Cheese

A panel consisting of 10 panelists (6 females and 4 males) with a mean age of 34 yr was used to perform 2 separate quantitative descriptive sensory analyses of the products obtained in each trial. Panelists were recruited and selected following the international standard ISO 8586–1 (ISO, 2012) by assessing their capacity to identify the 4 basic tastes (sourness, sweetness, bitterness, and saltiness), as indicated by Albenzio et al. (2013). Then, panelists were trained on the use of the scale (Stone and Sidel, 2004). Based on the available literature (Muir et al., 1995, 1996; Murray and Delahunty, 2000; Adhikari et al., 2003), panelists developed a specific vocabulary for Mozzarella cheese and agreed on a 19-attribute consensus list (Table 2) concerning appearance, odor/flavor, taste, and texture (3, 6, 3, and 7 attributes were identified, respectively). Three points of the scale (low, medium, and high intensity) were anchored to the reference material during the panel training to build a specific reference frame for assessor training (Table 2). The panel leader guided the assessors in selecting the most appropriate references for at least 2 anchor points of each sensory attribute (Albenzio et al., 2013). A quantitative descriptive analysis was used to evaluate the products (Lawless and Heymann, 2010) obtained in the first trial: 10-CTL and 10-FRS. Tests started at 1030 h and were conducted in sensory booths (ISO 8589; ISO, 1998). For the evaluation of appearance, booths were illuminated with white fluorescent lighting, whereas odor/flavor, taste, and texture attributes were assessed under red fluorescent lights to minimize color differences among samples. Samples (15–20 g cubes) were coded, randomized across panelists, replications, and samples, and served at 18°C. The intensity of each attribute was rated on 100 mm unstructured lines anchored at each end with 0 at the left end (attribute not perceived) and 100 at the right end (attribute perceived as very strong). Panelists drank a sip of water after each sample to make the conditions similar for each tasting. The interval between consecutive samples was roughly 10 min. The panelists received no information concerning the products under test and evaluated 2 replications of each product in one session. The same trained panel was used for the quantitative descriptive analysis of the products obtained in second trial (20-CTL and 20-FRS) as described in the previous one.

Consumer liking for mozzarella cheese was assessed only on the samples from the second trial using 94 untrained consumers (49 female and 45 male subjects) with an age ranging from 24 to 60 yr. Each consumer assessed two 15–20-g samples in random order in a controlled sensory analysis laboratory and in blind conditions. Participants were asked to express their overall liking for the 2 products. In addition, they were asked to express their liking for 3 specific aspects: texture, appearance, and taste/flavor. Participants used a 9-point hedonic scale with a central point corresponding to "neither pleasant nor unpleasant" (score = 5), a left end (score = 1) labeled as "extremely unpleasant" and a right end (score = 9) labeled as "extremely pleasant" (Kähkönen et al., 1996).

Statistical Analysis

Data from the 2 trials were separately analyzed by SAS, version 8.1 (SAS Institute Inc., Cary, NC). Two-way ANOVA per repeated measures (Mixed procedure) was used to test the effect of diet on milk yield and composition with treatment as nonrepeated factor and day of sampling as repeated factor. The cow variance was considered as random and used as the error term to test the main effect of diet. One-way ANOVA (Mixed procedure) was used to analyze data on chemical and fatty acid composition of mozzarella cheese. The batch of production was used as the error term to test the main effect of diet. For the variable color, 2-way ANO-

Table 2. Definition of the descriptive attributes used to assess mozzarella cheese

Descriptor	Definition
Appearance	
Color	Overall intensity of color (from white to ivory)
Brightness	Overall intensity of the light reflected from the external surface
Smoothness	Overall uniformity of the external surface
Odor/flavor	
Overall odor	Overall intensity of the odor
Overall flavor	Overall intensity of the flavor
Milk	Odor/flavor arising from milk at room temperature
Butter	Odor/flavor arising from butter at room temperature
Whey	Odor/flavor associated with whey
Yogurt	Odor/flavor associated with plain whole yogurt
Taste	
Salty	Fundamental taste associated with sodium chloride
Sour	Fundamental taste associated with citric acid
Sweet	Fundamental taste associated with sucrose
Texture	
Tenderness	Minimum force required to chew mozzarella samples: the lower the force, the higher the tenderness
Elasticity	Degree to which the original shape of a product is restored after compression between the teeth
Juiciness	Moisture released during mastication (low: saliva is absorbed by the product; high: liquids are abundantly released during mastication)
Cohesiveness	The degree to which a mozzarella sample holds together or adheres to itself while being chewed
Chewiness	Easiness to masticate the sample to a state pending swallowing
Screechy	Friction of the product against the teeth, typical of milk casein soon after hot water stretching

Table 3. Yield and composition (LSM ± SEM) of milk obtained from buffalo fed fresh sorghum (10-FRS and 20-FRS received 10 and 20 kg of fresh sorghum, respectively) and the corresponding control groups (10-CTL and 20-CTL, respectively) fed no fresh forages (n = 48)

Item	10-CTL	10-FRS	*P*-value	20-CTL	20-FRS	*P*-value
Yield, kg/animal per d	9.2 ± 0.28	9.3 ± 0.28	0.689	8.7 ± 0.26	9.1 ± 0.26	0.558
Chemical composition, g/kg						
Fat	88.1 ± 2.80	87.6 ± 2.80	0.895	90.6 ± 3.33	94.1 ± 3.33	0.240
Protein	49.9 ± 0.89	48.4 ± 0.89	0.261	50.7 ± 1.12	52.7 ± 1.12	0.202
Lactose	48.2 ± 0.43	48.9 ± 0.43	0.324	47.1 ± 0.71	47.1 ± 0.71	0.975

VA was performed as the effect of area of measurement (i.e., internal and external) was also examined.

Sensory profile data from each trial were separately subjected to a preliminary ANOVA to verify the reliability of the panel. In particular, the following fixed effects were assessed: diet (2 levels), replication (2 levels), and assessor (10 levels), and the corresponding first-order interactions. Subsequently, a mixed procedure was used to evaluate the fixed effect of diet (2 levels) using replication (2 levels) and assessor (10 levels) as random factors. A *t*-test was used to assess consumer likings expressed for the products obtained in the second trial. Statistical significance was declared at $P < 0.05$ and tendencies discussed at $P < 0.10$.

RESULTS AND DISCUSSION

Milk Production and Chemical Composition and Color of Mozzarella

Table 3 shows milk yield and composition of the 2 CTL groups, and the corresponding 2 experimental groups fed the diets containing fresh sorghum at 13.4 (10-FRS) and 26.5% DM (20-FRS). No effects of diet were observed for any parameters. Similarly, no significant differences were observed between CTL compared with 10-FRS and 20-FRS mozzarella for chemical composition, even if fat content tended ($P = 0.103$) to be higher in 20-FRS than in CTL mozzarella (Table 4). No differences were observed between groups FRS and the corresponding control groups in terms of mozzarella instrumental color. This result was expected because the white color is a basic requirement of buffalo mozzarella related to the physiology of these animals (Jana and Mandal, 2011). Differences were found for lightness, redness, and yellowness of internal and the external area of mozzarella ($P < 0.001$) due to their different texture (data not shown).

Fatty acid profile of mozzarella cheese under the different diets is presented in Table 5. Fatty acid composition of CTL and 10-FRS mozzarella did not differ, with the exception of C6:0 and C16:1 ($P < 0.05$) being lower in 10-FRS. In the second trial, 20-FRS mozzarella had lower values of C4:0, C6:0, C8:0 ($P < 0.001$), and C10:0 ($P < 0.05$), and higher contents of C18:1n-9 *cis*, C18:3n-3, C18:1 *trans*-11, CLA *cis*-9,*trans*-11, C22:0 ($P < 0.001$), C18:0, and C20:0 ($P < 0.05$). A tendency ($P \leq 0.10$) was also observed for C14:0 and C16:0 to be lower. As consequences, 20-FRS mozzarella presented higher levels of PUFA and MUFA, lower percentages of SFA, and a better value of atherogenic index ($P < 0.001$).

Overall, although the fatty acid profile in mozzarella cheese fat was not dramatically modified, feeding 20 kg of sorghum/animal (group 20-FRS) allowed to triple the contents of C18:3, and markedly increased the content of CLA and PUFA. It is well established that fat from grazing animals or fed high fresh forage diets have higher proportions of PUFA and particularly n-3 fatty acids, versus conventional cheese because forages are naturally rich sources of C18:3n-3 (Ellis et al., 2006; Lourenço et al., 2008). Moreover, long-chain UFA can

Table 4. Chemical composition and color of mozzarella cheese (LSM ± SEM) obtained from buffalo fed fresh sorghum (10-FRS and 20-FRS received 10 and 20 kg of fresh sorghum, respectively) and the corresponding control groups (10-CTL and 20-CTL, respectively) fed no fresh forages (n = 9)

Item	10-CTL	10-FRS	*P*-value	20-CTL	20-FRS	*P*-value
Chemical composition, g/kg						
Moisture	491.7 ± 0.90	487.5 ± 0.90	0.755	492.4 ± 1.11	479.8 ± 1.11	0.470
Fat	276.0 ± 0.28	278.8 ± 0.28	0.505	276.2 ± 0.36	287.0 ± 0.36	0.103
Protein	208.7 ± 0.43	204.2 ± 0.43	0.518	206.9 ± 0.67	201.3 ± 0.67	0.587
Color						
L (lightness)	95.5 ± 0.63	95.2 ± 0.63	0.749	93.3 ± 2.00	93.6 ± 2.00	0.931
a* (red-green)	−2.7 ± 0.14	−2.3 ± 0.14	0.132	−2.7 ± 0.21	−2.5 ± 0.21	0.532
b* (yellow-blue)	8.6 ± 0.31	8.5 ± 0.31	0.746	8.7 ± 0.47	7.8 ± 0.47	0.247

inhibit mammary gland synthesis of short-chain fatty acids in milk fat, leading to a reduction of SFA (Grummer, 1991). The higher intake of C18:3 can result in an increment of C18:1 *trans*-11 produced in the rumen by biohydrogenation (Bauman et al., 2000) and, as a consequence, in an increment of CLA *cis*-9,*trans*-11 by the action of Δ^9-desaturase (Kay et al., 2004). It has been also suggested that green grass, due to the high concentrations of soluble nitrogen, sugars, and soluble fiber, can enhance the growth of rumen bacteria producing CLA *cis*-9,*trans*-11 or blocking biohydrogenation of C18:1 *trans*-11 in the rumen, thus leading to its accumulation and availability for conversion to CLA *cis*-9,*trans*-11 in the mammary gland via Δ^9-desaturase (Kelly et al., 1998; Nudda et al., 2005). While numerous studies investigated fatty acid composition of bovine milk fat, literature on buffalo is still limited (Varricchio et al., 2007; Zotos and Bampidis, 2014; Pegolo et al., 2017). The use of flax seeds determined changes of buffalo fat similar but larger than those observed in the present study (Santillo et al., 2016). In this respect, Dewhurst et al. (2006) indicate that fresh forages are less efficient at altering milk fatty acids than fats or concentrates. Accordingly, in this study the inclusion of 10 kg of fresh sorghum was unable to modify fatty acid composition of mozzarella, whereas in dairy cattle (Couvreur et al., 2006) the inclusion of fresh forage at 30% of dietary DM determined results similar to those we observed for the 20-FRS diet. Nevertheless, whereas oilseed or fat supplementation tend to increase the cost of the feeding ration, fresh forage has the potential to lower the feeding costs (Borreani et al., 2013). In addition, it may be conveniently used for quality-identified products, such as Mozzarella di Bufala PDO cheese, as for these products any change of raw materials are restricted or even prohibited, whereas the origin of forage may give the basis for the "terroir" notion (Verdier-Metz et al., 2005). Moreover, fresh forage feeding may allow product differentiation (Tempesta and Vecchiato, 2013) and increase mozzarella liking as a consequence of the increased expectations induced by the modified process characteristics (Napolitano et al., 2010). According to Vecchio et al. (2016), a large share of consumers would be interested in mozzarella PDO with a better fatty acid profile, but most of them expect these added-value products without substantial extra costs.

Sensory Properties

The fixed ANOVA separately conducted for each trial showed that in both cases the interactions of assessor × replication and assessor × diet were not significant. In

Table 5. Fatty acid composition (% of total fatty acids) of mozzarella cheese (LSM ± SEM) obtained from buffalo fed fresh sorghum (10-FRS and 20-FRS received 10 and 20 kg of fresh sorghum, respectively) and the corresponding control groups (10-CTL and 20-CTL, respectively) fed no fresh forages (n = 9)

Fatty acid	10-CTL	10-FRS	*P*-value	20-CTL	20-FRS	*P*-value
C4:0	4.41 ± 0.13	4.50 ± 0.13	0.661	5.09 ± 0.10	2.65 ± 0.10	<0.001
C6:0	3.14 ± 0.08	2.82 ± 0.08	0.048	3.00 ± 0.04	1.51 ± 0.04	<0.001
C8:0	1.30 ± 0.06	1.09 ± 0.06	0.070	1.24 ± 0.02	0.91 ± 0.02	<0.001
C10:0	3.81 ± 0.19	3.78 ± 0.19	0.927	3.34 ± 0.02	3.45 ± 0.02	0.023
C12:0	5.65 ± 0.11	5.48 ± 0.11	0.336	5.44 ± 0.05	5.33 ± 0.05	0.255
C13:0	0.56 ± 0.06	0.46 ± 0.06	0.3348	0.155 ± 0.09	0.159 ± 0.09	0.770
C14:0	14.90 ± 0.14	15.30 ± 0.14	0.166	14.69 ± 0.11	14.26 ± 0.11	0.059
C14:1	0.90 ± 0.07	0.94 ± 0.07	0.674	1.27 ± 0.01	1.29 ± 0.01	0.676
C15:0	2.73 ± 0.17	2.59 ± 0.17	0.615	2.04 ± 0.06	2.16 ± 0.06	0.200
C16:0	34.38 ± 0.32	34.63 ± 0.32	0.601	34.23 ± 0.14	33.63 ± 0.14	0.070
C16:1	3.1 ± 0.09	2.7 ± 0.09	0.050	3.30 ± 0.04	3.23 ± 0.04	0.3037
C17:0	0.89 ± 0.04	0.79 ± 0.04	0.136	0.77 ± 0.07	0.70 ± 0.07	0.556
C17:1	0.48 ± 0.04	0.47 ± 0.04	0.925	0.29 ± 0.01	0.31 ± 0.01	0.672
C18:0	7.61 ± 0.16	8.02 ± 0.16	0.138	8.80 ± 0.11	9.87 ± 0.11	0.022
C18:1n-9 *cis*	13.23 ± 0.38	13.44 ± 0.38	0.717	13.60 ± 0.14	16.40 ± 0.14	<0.001
C18:1 *trans*-11	0.31 ± 0.02	0.34 ± 0.02	0.410	0.50 ± 0.02	0.77 ± 0.02	0.0004
C18:2n-6 *cis*	1.72 ± 0.15	1.73 ± 0.15	0.976	1.43 ± 0.02	1.47 ± 0.02	0.369
C18:3n-3	0.24 ± 0.03	0.20 ± 0.03	0.313	0.26 ± 0.01	0.75 ± 0.01	<0.001
CLA *cis*-9,*trans*-11	0.34 ± 0.02	0.37 ± 0.02	0.459	0.30 ± 0.02	0.74 ± 0.02	<0.001
C20:0	0.15 ± 0.01	0.20 ± 0.01	0.182	0.16 ± 0.01	0.25 ± 0.01	0.002
C22:0	0.12 ± 0.01	0.16 ± 0.01	0.121	0.01 ± 0.07	0.02 ± 0.07	0.0007
Saturated	79.70 ± 0.34	79.86 ± 0.34	0.801	79.01 ± 0.17	75.01 ± 0.17	<0.001
Unsaturated	20.31 ± 0.35	20.19 ± 0.35	0.827	20.95 ± 0.11	24.95 ± 0.11	<0.001
MUFA	18.0 ± 0.44	17.9 ± 0.44	0.8680	18.9 ± 0.17	22.41 ± 0.17	<0.001
PUFA	2.23 ± 0.17	2.29 ± 0.17	0.977	2.00 ± 0.03	2.96 ± 0.03	<0.001
ATI[1]	4.95 ± 0.12	5.0 ± 0.12	0.5654	4.7 ± 0.05	3.84 ± 0.05	0.0005

[1]Atherogenic index [C12:0 + (4 × C14:0) + C16:0]/UFA.

Table 6. Sensory profile of mozzarella cheeses (LSM ± SEM) obtained from buffalo fed fresh sorghum (10-FRS and 20-FRS received 10 and 20 kg of fresh sorghum, respectively) and the corresponding control groups (10-CTL and 20-CTL, respectively) fed no fresh forages

Attribute	10-CTL	10-FRS	*P*-value	20-CTL	20-FRS	*P*-value
Appearance						
Color	60.35 ± 1.45	57.95 ± 1.45	0.2679	74.15 ± 2.43	74.95 ± 2.43	0.831
Brightness	65.70 ± 1.94	67.45 ± 1.94	0.5374	65.30 ± 2.43	72.15 ± 2.43	0.074
Smoothness	67.70 ± 0.86	66.80 ± 0.86	0.4764	61.55 ± 2.67	74.00 ± 2.67	0.008
Odor/flavor						
Overall odor	59.15 ± 2.38	58.20 ± 2.38	0.7836	81.45 ± 1.74	74.80 ± 1.74	0.022
Overall flavor	56.60 ± 2.01	60.45 ± 2.01	0.2052	65.15 ± 2.08	57.70 ± 2.08	0.030
Milk	55.05 ± 1.72	54.20 ± 1.72	0.7335	70.05 ± 2.05	59.25 ± 2.05	0.004
Butter	49.90 ± 2.64	49.05 ± 2.64	0.8242	48.90 ± 3.12	51.75 ± 3.12	0.533
Whey	45.25 ± 2.43	42.80 ± 2.43	0.4927	24.60 ± 2.04	17.45 ± 2.04	0.032
Yogurt	41.70 ± 2.77	40.05 ± 2.77	0.6828	20.60 ± 1.70	21.35 ± 1.70	0.762
Taste						
Salty	43.55 ± 1.66	48.10 ± 1.66	0.0820	39.20 ± 3.10	36.00 ± 3.10	0.483
Sour	42.30 ± 2.28	41.95 ± 2.28	0.9158	33.10 ± 1.38	16.80 ± 1.38	<0.0001
Sweet	23.55 ± 1.39	22.15 ± 1.39	0.4929	19.40 ± 1.11	28.75 ± 1.11	<0.0001
Texture						
Tenderness	67.10 ± 2.65	67.20 ± 2.65	0.9792	55.35 ± 2.57	78.70 ± 2.57	<0.0001
Elasticity	58.85 ± 2.60	63.95 ± 2.60	0.1952	68.65 ± 2.17	58.80 ± 2.17	0.009
Juiciness	70.55 ± 1.71	72.00 ± 1.71	0.5611	47.20 ± 2.05	69.60 ± 2.05	<0.0001
Cohesiveness	66.10 ± 1.81	67.95 ± 1.81	0.4861	77.25 ± 2.00	62.35 ± 2.00	0.0003
Chewiness	67.00 ± 2.06	69.80 ± 2.06	0.3584	91.15 ± 2.89	66.65 ± 2.89	<0.0001
Screechy	61.35 ± 2.62	64.00 ± 2.62	0.4909	77.90 ± 2.13	63.20 ± 2.13	0.0007

addition, the 2 products did not change their sensory properties across replications as the interaction replication × diet was not significant. Therefore, a first relevant result of the present study was the development of a specific reference frame for the training of panelists to be used in the sensory analysis of mozzarella cheese. Second, we noted that the inclusion of 10 kg of fresh forage per animal was unable to change the sensory profile of mozzarella cheese, whereas when 20 kg of sorghum was used, most of the attributes were able to discriminate the product obtained by feeding the buffalo fed fresh forage from that obtained by feeding no fresh forage (Table 6). In particular, in the second trial the trained panel perceived the mozzarella 20-FRS to have a lower overall odor, overall flavor ($P < 0.05$), milk ($P < 0.01$), and whey odor/flavor ($P < 0.05$). Previous studies obtained controversial results in terms of odor/flavor with some attributes increasing when animals were kept on pasture, other attributes decreasing, and no effect on odor intensity (see Coulon et al., 2004, for a review). More recently Coppa et al. (2011) recorded higher intensities of overall odor and aroma in Cantal cheese obtained from grazing cows as compared with the indoor control system, whereas Agabriel et al. (2004) noted a decrement of odor intensity as a consequence of the consumption of fresh forage in grazing cows. However, none of these authors included *pasta filata* cheese in their studies, and process-related factors could interact with the feeding regimen in affecting cheese quality. Conversely, Esposito et al. (2014) found higher intensities of most odor/flavor attributes describing Caciocavallo (a semi-hard *pasta filata* cheese) obtained in winter when the cows were kept indoors and received hay and concentrate compared with the same cheese manufactured in spring when the animals were managed outdoors and pasture represented the primary feeding source. In addition, mozzarella is a fresh product consumed within few hours from the end of the production process, whereas in all the other studies cheese was always ripened for at least 3 mo and ripening can markedly affect cheese sensory profile (e.g., Coppa et al., 2011). In particular, the higher content of UFA in cheese produced from grazing animals can affect the development of odor/flavor active compounds only after an adequate ripening (Farruggia et al., 2014), although some cheese flavor molecules may also originate from ruminal enzymatic degradation of certain UFA (Coulon et al., 2004). Nevertheless, other milk/cheese components (e.g., terpenes) should be investigated to identify molecules and biological processes responsible for odor/flavor differences induced by fresh forage feeding.

In agreement with previous studies on *pasta filata* cheese (Carpino et al., 2004; Bonanno et al., 2013; Esposito et al., 2014), in terms of taste we observed an increased intensity of the attribute sweet and a decreased intensity of the attribute sour ($P < 0.001$) when fresh forage at 20 kg/animal was included in the diet as compared with the control products. Other authors observed similar results for the attribute sour (Agabriel et al., 2004; Coulon et al., 2004), whereas Frétin et al.

Table 7. Hedonic test (mean ± SE) of Mozzarella cheese obtained from buffalo either fed fresh sorghum (20-FRS) or not (20-CTL)

Liking[1]	20-CTL	20-FRS	P-value
Overall appearance	6.51 ± 0.17	6.85 ± 0.17	0.158
Taste/flavor	5.69 ± 0.17	5.87 ± 0.17	0.460
Texture	6.03 ± 0.18	6.02 ± 0 18	0.960
Overall liking	6.56 ± 0.15	6.58 ± 0.15	0.923

[1]Mean liking based on a 9-point hedonic scale from "extremely unpleasant" (1) to "extremely pleasant" (9).

(2017) found opposite results in uncooked cheese but only when pasteurized milk was used.

The 2 experimental products were also discriminated based on all of the texture attributes, as 20-FRS showed higher intensities for tenderness and juiciness and lower intensities for elasticity, cohesiveness, chewiness, and the attribute screechy as compared with 20-CTL ($P < 0.001$). The effect of fresh forage feeding on texture attributes of mozzarella can be explained on the basis of the lower SFA content and a fat content that tended to be higher. Both chemical characteristics can contribute to make the cheese softer and stickier (e.g., Coppa et al., 2011; Frétin et al., 2017).

Unsurprisingly, the color of mozzarella was unaffected by the diet in both trials, whereas 20-FRS showed a higher uniformity of the external surface and thus was assessed as smoother in terms of appearance in comparison with the corresponding control group ($P < 0.01$). Numerous authors observed changes in cheese sensory color as a consequence of the ingestion of fresh forage and the related β-carotene content (e.g., Esposito et al., 2014). However, these changes could not be observed in buffalo mozzarella cheese, as buffalo milk does not contain detectable amounts of β-carotene (Cerquaglia et al., 2011), due to a more efficient liver enzymatic conversion system of the β-carotene into retinol (Mora et al., 2000).

Although both products were rated above the neutral point (i.e., 5 = neither pleasant nor unpleasant), the panel composed of untrained and uninformed consumers did not show any preference ($P > 0.05$) in terms of appearance, taste/flavor, texture, and overall liking (Table 7). These results indicate that consumers were unable to perceive the subtle albeit significant sensory differences detected by the trained panel.

CONCLUSIONS

This study demonstrated that the inclusion of fresh sorghum in a buffalo TMR at least 26.5% on a DM basis is able to modify the fatty acid composition of buffalo mozzarella cheese. The sensory properties of mozzarella were also modified, whereas no effect on consumer blind acceptance were found. Lower amount of fresh forages (i.e., 13.4% on DM basis) did not have any effects on fat composition or sensory properties of mozzarella. We conclude that, although there are other more effective feeding strategies to modify mozzarella fatty acid profile, fresh-cut forage feeding can represent a low-cost technique to increase the PUFA and CLA content of mozzarella. Further studies are needed to verify whether the information concerning fresh forage feeding may increase mozzarella actual liking, thus providing a tool for product differentiation.

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