Doctoral thesis

IMPROVEMENT OF FORAGE YIELD TO IMPROVE DAIRY PRODUCT QUALITY: MYCORRHIZAL FUNGI APPLICATION AND DIFFERENTIATION OF FORAGE CONSERVATION METHODS

by

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ABBREVIATIONS

µm	Micro liter
A	Farm A
a.m.	After morning
ADF	Acid detergent fiber
ADL	Acid detergent lignin
AM	Arbuscular mycorrhizal
AMF	Arbuscular mycorrhizal fungi
B	Farm B
Br	Bromine
°C	Centigrade
C	cheese
Ca	Calcium
CC	Curd after coagulation
Cl	Chloride
cm	centimeter
CS	Curd end of acidification
Cu	Cupper
d	day
DM	Dry matter
EU	European Union
FA	Fatty acid
Fe	Iron
G	gram
h	hour
H	Hay
ha	hectare
i.e.	More examples
K	Potassium
Kcal	Kilogram calorie
kg	kilogram
L	sample
LSD	Least square degree
m	Meter
M-	Not mycorrhizal
M+	mycorrhizal
MBC	Mozzarella di Bufala Campana
Mg	Magnesium
ml	milliliter
MM-	Not Mycorrhizal maize
MM+	Mycorrhizal maize
Mn	Manganese
MS-	Not Mycorrhizal sorghum
MS+	Mycorrhizal sorghum
N	Nitrogen
n	Number
n-3	Omega 3
Na	Sodium
NDF	Neutral detergent fiber
NPN	Non-protein nitrogen
NS	Not significant
NWC	Natural whey culture
P	Phosphorus
PDO	Protected of designated origin
PGI	Protected geographical indication
pH	power of hydrogen
S	Sulfur
S	Silage
SEM	Standard error mean
SH	Soxhlet-Henkel degrees
TFP	Traditional food product
TMR	Total mixed ration
TSG	Traditional specialty guaranteed
UFL	Unitè foraggere Lait
vs	Versus
Zn	Zinc

ABSTRACT

The research is focused on forages production as a factor influencing the efficiency of buffalo and cattle farming and the quality of dairy products. The study is divided into two parts, which focus on forage production and dairy food quality, respectively.

1. The effects of the bio-stimulant vesicular-arbuscular mycorrhizal fungi on yield traits of sorghum and maize crops under low nutrient supply were examined. Moreover, the effects of mycorrhizal forage on milk yield of buffaloes and cows were surveyed. Study was carried on an irrigated plain farm (maize trail) and a dryland mountain farm (sorghum trail). *Zea mais* and *Sorghum sudanese* seeds were inoculated with a commercial product based on the mycorrhizal fungi *Glomus* spp. The control crops had the full dose of N and P, whereas the mycorrhizal ones grown under half dose of N and without P supply. At harvesting, forages were ensiled and fed to lactating buffaloes (maize) and dairy cows (sorghum). The results indicate that, for both forage crops studied, no reductions of forage yield were found associating the diminished supply of fertilizers with the use of mycorrhizal fungi. The forage produced by using mycorrhizal treatment showed better chemical characteristics if harvested at right phenological stage. There are no significant effects on quantity and quality of the milk produced by both buffaloes and cows.

2. This research line examined the influence of forage preservation method on cheese quality and it is branched in two trials. The first one compares the quality of the traditional Caciocavallo di Castelfranco obtained by using hay based or silage based diets. Forty-four lactating Holstein–Friesian cows were divided into Silage and Hay groups. On three consecutive days, bulk morning milk was collected separately and used to produce on farm Caciocavallo di Castefranco cheese. The analysis were carried out on cheese at 30, 60 and 90 d of ripening. No defects were recorded (no blowing or poor taste and odor) in cheese produced from the two diets, and this appears to be due to the good quality of silage. The study highlighted that the use of sorghum silage sensitively modify the sensory and organoleptic characteristics of the cheeses. Many effects are due to the presence in the raw milk of compounds directly induced by feeding (carotenes and terpenes). However, several of these effects appeared different at different stages of during ripening as a results of interaction between these molecules and the formation of new compounds in cheese during ripening. Consumers perceived differences between cheeses produced with the two diets at extreme ripening times.

A second trial was undertaken to evaluate the effect of feeding fresh forage on quality "*Mozzarella di Bufala Campana*" PDO cheese. Two homogenous groups of 16 lactating buffaloes were fed two total mixed rations containing or not 20 kg of fresh sorghum. On three days, milk from the two groups (on average 200 kg) were used to produce mozzarella PDO cheese. The inclusion of at least 20 kg of fresh forage in the diet of lactating buffaloes allowed an improvement of the fatty acid profile of the mozzarella with an increase of the content of PUFA and CLA. While modifying the sensory profile and the volatile fraction of the mozzarella, the use of fresh fodder did not change the product acceptability of *Mozzarella di Bufala Campana*. Efforts should be made by producer to signal the quality of this product to the consumer to improve its recognition.

Keywords: mycorrhizal inoculum, maize, sorghum, hay, silage, fresh forage caciocavallo cheese mozzarella, fatty acid, sensory properties, volatile compounds, consumer liking

OUTLINE OF RESEARCH

Forages are the basis of diets for dairy cows, and a focus on quality forage is essential to productivity and profitability of dairy farms. The research is focused on forages production as a factor influencing the efficiency of buffalo and cattle farming and the quality of dairy products. The study consists of two lines:

Chapter 1. ***Mycorrhizal fungi application on forage crop: effect on yield and animal performances.*** In the last years, it has been a growing interest in the use of natural and environmentally friendly bio-stimulants in agricultural field. In particularly, many studies aimed to develop the use of microorganism growth- promoters in order to increase crop yield and decrease the dependence on synthetic fertilizers. Arbuscular Mycorrhizal Fungi form mutualistic relations with plant roots. In these relationships, the fungus derives photosynthate from the host plant, which, in turn, gains greater quantities of nutrients from the association. Aim of the research line was to evaluate, under the usual conditions of cropping and farming of two experimental sites, the effects of mycorrhizal fungi on forage production under low nitrogen and phosphorus supply. Moreover, the influence of mycorrhizal forages on milk yield and quality was examined.

Chapter 2. ***Effect of different forage preservation methods on cheese quality***. The starting premise of the study was that feeding can influence microbiological profile, chemical composition, nutritive value, and sensorial attributes of dairy products. It is known that forages are the major and the cheapest fatty acid sources in the ruminant ration. Moreover they have the unique ability to synthesize the α- linoleic acid which is the building block of the n -3 series of essential fatty acids. The use of fresh forage in animal diet can increase the rate of unsaturated fatty acids deposition in milk and cheese and it can affect color and other sensorial properties of dairy products. Determining the relationship between green forages and sensory properties of dairy products getting to be more important, especially for the products that have been granted PDO status.

On the other hand, although the utilization of silage as feed for dairy cow is widespread, the effects of silages on cheese quality (chemical, physical, microbiological and technological characteristics) remain not completely clear. Moreover, when silages are poorly preserved the presence of butyric spores may create major quality problems for the ripened cheese.

Therefore, this part of the research aimed to investigate the effect of different forage conservation methods on dairy products quality. In particular, it was investigated (a) the effects of fresh forage feeding on mozzarella cheese quality, and (b) the effects of diets based on silage or hay on characteristics of caciocavallo cheese.

CHAPTER 1 – EXPERIMENT A
MYCORRHIZAL FUNGI APPLICATION ON FORAGE CROP: EFFECT ON YIELD AND ANIMAL PERFORMANCES

1a. THE MYCORRHIZAL FUNGI

The term "mycorrhiza", used the first time more than 100 years ago by Frank, a Germany scientist, indicates a mutualistic association between a group of soil fungi and higher plants (Habte, 2000). Mycorrhiza improve plant nutrition uptake by extending its root system, whereas plant provides to the fungi carbohydrates and organic components that are essential for fungal life cycle (Bonfante and Genre, 2008).

Several kinds of mycorrhizal associations have been described according to the morphologic characteristics, and plant and fungal taxa implicated (Gianinazzi-Pearson, 1996). The most diffused and useful classification distinguishes between endomycorrhizal and ectomycorrhizal associations (Siddiqui et al., 2008). In the endomycorrhizal association fungus penetrates into the root cortex both intercellularly and intracellularly, while in ectomycorrhizal association fungus penetrates only intercellularly in the root cortex (Siddiqui et al., 2008).

The endomycorrhizas are also indicated as vesicular arbuscular mycorrhizal fungi, since they form a mycelium bearing vesicles and arbuscules in the root cortex and large resting spores in the soil around infected roots (Smith and Read, 2010).

The arbuscular mycorrhizal fungi (AMF) are classified into three classes (Archaeosporomycetes, Glomeromycetes, and Paraglomeromycetes), and the five orders: Archaeosporales (e.g. *Geosiphon pyriformes, Archaeospora trappei*), Diversisporales (e.g. *Scutellospora calospora*, *Acaulospora laevis, Entrophospora infrequens*), Gigasporales (e.g. *Gigaspora margarita*, *G. rosea*), Glomerales (e.g. *Glomus intraradices*, *G. mosseae*, *G. geosporum*) and Para glomerales (e.g. *Paraglomus occultum, P. laccatum*) (Parniske, 2008).

However, most AM fungi that can be detected in natural ecosystems have not been cultured and it is possible that many have a more restricted host range than 'generalists', such as *Glomus mossae* or *Glomus intraradices*, which are intensively investigated since they are easily cultured (Parniske, 2008).

1.1a. Roles of arbuscular mycorrhizal fungi on plant life and nutrition

About 80% of all terrestrial plants, including many important agricultural crop species such as soybean, corn, rice, and wheat, form arbuscular mycorrhizal association (Bucking et al., 2012; Smith and Read, 2010; Quilambo, 2004). AMF colonize the host roots and promote plant growth under relatively harsh conditions by expanding the surface area via production of extensive hyphae (Ocak and Demir, 2012).

The symbiosis has many important and positive effects on plant and soil lifecycle. It influences plant biodiversity (van der Heijden et al., 1998), improves nutrient uptake, increases tolerance to salinity and to heavy metals (Al-Karaki et al., 2001; Díaz et al., 1996; Feng et al., 2002; Mohammad et al., 2003; Shetty et al., 1995), helps to protect against of the pests (e.g. nematodes) and fungal pathogens (Azcón-Aguilar and Barea, 1997), decreases drought stress (Augé, 2001), and affects the fitness of plants in polluted environments (Hildebrandt et al., 1999). In addition, mycorrhizae have been shown to play an important role in maintaining soil aggregate stability (Degens et al., 1996; Tisdall, 1994; Tisdall et al., 1997). Thus, mycorrhizal fungi influence land life directly or indirectly (Schüβler et al., 2001).

The most consistent and important nutritional effect of AMF is the improvement of uptake of immobile nutrients. AM fungi absorb N, P , K, Ca, S, Fe, Mn, Cu, Zn, Mg, Cl and Br minerals from the soil and translocate this nutrients to the plants (Clark and Zeto, 2000; Gosling et al., 2006; Haselwandter et al., 1988; Hodge et al., 2001; Mohammadi, Khosro, et al., 2011; Robb, 2013; Smith and Read, 2010). The enhanced uptake of P is generally regarded as the most important benefit that AMF provide to host plant (Graham, 2000; Smith and Read, 2010; Thompson, 1994). AMF can play a significant role in crop P nutrition, increasing total uptake and in some cases P use efficiency (Koide, 1991). This may be associated with increased crop growth and yield (Koide et al., 2000; Vosátka, 1995).

Another advantage of mycorrhizal symbiosis to associated plants is the reduction of plants' external P requirement. Consequently, plants grown in association with AMF can grow with only a fraction of the P required by plants lacking mycorrhizal association. The ability of AMF to reduce plants' external P requirement has an important environmental benefit. High levels of P in soils can result in P enrichment of water that causes eutrophication due to excessive development of algae, cyanobacteria, and aquatic plants (Culley et al., 1983; Sharpley et al., 1992). AM fungi, therefore, are an important component of nutrient management programs that aim to reduce environmental pollution (Habte and Osorio, 2001).

2a. AIMS

In recent years, great importance has assumed the use on field of bio-inoculants, which are proposed as a new factor of sustainable crop production, much so that they were included among the "special fertilizers" (Baruffa et al., 2008).

Arbuscular mycorrhizal fungi form mutualistic associations with the roots of the majority of terrestrial plant species. In these relationships, the fungus derives photosynthate from the host plant, which in turn gains greater quantities of nutrients from the association. In addition, mycorrhizal fungi can increase the efficacy of root water absorption. Therefore, the use of arbuscular mycorrhizal fungi in forage crop could reduce nutrient supply and help plant to overcome water stress during water deficiency.

Aim of this research was to evaluate, under low supply nutrient conditions the effects of mycorrhizal fungi on forage production. Moreover, the effects of mycorrhizal forages on milk yield and quality were examined.

The study consisted of two trials carried out in two experimental sites, which are separately presented:

A. Maize trial

A.1 Yield of maize produced by using AM fungi under low Nitrogen and Phosphorus supply.

A.2 Quality of maize silage produced using AM fungi

A.3 Milk production of buffalo cows fed diet containing mycorrhizal maize.

B. Sorghum trial

B.1 Yield of sorghum produced by using AM fungi under low Nitrogen and Phosphorus supply.

B.2 Quality of sorghum haylage produced using AM fungi

B.3 Milk production of dairy cows fed diet containing mycorrhizal sorghum haylage.

3a. MATERIAL AND METHODS

3.1a. Study Sites

The study was carried on two heterogeneous agriculture contexts, Sele river plan and Fortore beneventano, in each of which a representative dairy farm was chosen. These areas, which differ substantially in terms of environmental heterogeneity and rural development, were selected according to the presence of a quite large livestock sector.

The **Sele river plain** is located in the south-west part of Campania region. The soils are mainly clay-loam and silty-clay, volcanic origin. The climate is Mediterranean maritime (Csa climate, sensu Koppen) with a relatively dry summer (84 mm) and a mean annual rainfall of 988 mm. The monthly temperature ranges between the minimum of 9.0 ° C (January) to the maximum of 23.6 °C (August). The main crops are maize, forages, vegetables (artichokes, onion, cucurbits, pepper, tomato, lettuce), and orchards (peaches, apricot, pears). Livestock farming is focused on dairy buffalo (Italian Mediterranean water buffalo) to produce Mozzarella di Bufala Campana PDO cheese. A buffalo dairy farm, afterwards named A (40°31'N 14°57'E), was chosen as experimental site. Other characteristics of the farm are summarized in Table 1a.

The **Fortore area** is a part of the Apennine mountain range of southern Italy, on the borders between Puglia, Campania and Molise Regions. The area is mainly mountainous and hilly, covering between 400 and 950 m above sea level. The flysch substratum makes the area particularly sensitive to the risk of soil erosion. The area presents Mediterranean sub continental climate conditions (Csb climate, sensu Köppen). The mean annual rainfall is 1001 mm, but precipitations are concentrated in autumn and winter. Therefore, the supply of water during the warm (15-20 °C) summer is not sufficient for crops with high water needs such as maize. Land is primarily used for cereal and forage crops. Frequent is the lack of irrigation water. Livestock farming is focused on sheep and cattle. Milk is used for human consumption, either fresh or as cheese. A dairy farm, afterwards named B (41°16'N 15°05'E), was chosen as experimental site (Table 1a).

3.2a Forage Species and Mycorrhizal Treatment

Due to the different environmental and agricultural context of the experimental sites, two different forages were chosen as the focus for the research. Maize (Zea mais, cv Indaco class FAO 600, Limagrain®, Busseto PR, Italy) was chosen in Sele plain, because it is the most widely forage crop in intensive dairy livestock farming in plains. Sorghum (medium–

early hybrid *Sudangrass x Sudangrass* Hermes® Padana Sementi Elette, Tombolo, PD, Italy) was chosen in Fortore mountain, since it is suitable for both ensiling and haymaking and has less requirements in nutrient and irrigation water. Maize was irrigated, sorghum grew under rainfed conditions.

For both species, the same mycorrhizal complex (Aegis®, Italpollina, Rivoli Veronese, VR, Italy) containing spores of the mycorrhizal fungi *Glomus intraradices* (700 spores/g) and *Glomus mossae* (700 spores/g) was used. The product was formulated as wettable power to be directly sprayed on seeds. In May 2014, sorghum and maize seeds were inoculated on farms with *Glomus* spp.. The complex, mixed with a green adhesive powder and a starter solution containing amino acids and peptides of vegetal origin, was sprayed on the seeds in a cement mixer at the dose of 20 g inoculum/kg seed (i.e. 14 spores/g seed). The mycorrhizal (M+) and not mycorrhizal (M-) seeds were stored on farm in paper bags at room temperature until used.

3.3a Maize

Mycorrhizal (MM+) and not mycorrhizal (MM-) maize seeds were sown in farm A on June, 7 2014, arranging 1.5 ha plots with 2 replications/treatment. Overall, the maize forage production was carried out on a 6 ha area. The plots were situated in the same field with a corridor 3 m wide between them. Seeds were planted with a pneumatic seed drill. The control (MM-) treatment received the full dose of P and N (i.e. N 250 kg ha^{-1}, P 100 kg ha^{-1}) calculated on the basis of both uptake (3.9 kg N Mg^{-1} yield, and 1.5 $P_2O_5Mg^{-1}$ yield) and expected yield (65.0 Mg ha^{-1}) suggested by the Regional Department of Agriculture (Mori and Di Mola, 2012). The doses adopted were similar to those used by the farmers in local practices. The MM+ treatments received only half dose of N (125 kg ha^{-1}). N was applied half at seeding, together with P supply, and half at 4-leaves stage. Maize was irrigated as and when required, for a total irrigation volume of 600 m^3/ha. MM+ and MM- forages were harvested on September, 25 2014, and put into 4 silobags, one per plot. Due to the bad weather, maize was at early wax ripening phase at harvest. Forages were chopped at 1.50 cm theoretical length of cut using the same harvester. Quality of MM- and MM+ silages was determined 4 months after ensiling. Thereafter, a feeding trial was undertaken.

3.3.1a Feeding trial

Forty lactating buffalo cows were divided into MM- and MM+ groups balanced for parity (2.2 ± 0.6 vs. 2.3 ±0.6), days in milk (108±21 vs. 111±20 d) and milk production (11.1±3.8 vs. 11.3±3.4 kg/head/d). Groups were housed in two adjacent free stall pens with

concrete floor and equipped with feed manger, automatic drinker, and outdoor paddock. Animals were handled in similar way in terms of feeding and management and were milked twice daily (05:00 and 17:00). The groups were fed 2 total mixed rations (TMR) differing for the use of mycorrhizal (MM+ group) or not mycorrhizal (MM- group) maize silage (Table 8a). Cows were fed once daily (07:00 h) in amount to provide approximately 10% orts for ad libitum consumption. After 10 d of adaptation, during 6 consecutive d, yield of each buffaloes was determined and milk sampled. Amount of feed offered and refused was measured daily on group basis.

3.4a Sorghum

In farm B, the sorghum trial was carried out in similar manner to maize experiment. Mycorrhizal (MS+) and not mycorrhizal (MS-) sorghum were sown, on June, 3 2014, on 4 plots, 1.5 ha each. The SM- received the full dose of P and N (i.e. N 120 kg ha-1, P 50 kg ha^{-1}) calculated as previous described (3.0 kg N and 1.0 kg P_2O_5 Mg^{-1} yield; 40.0 Mg ha^{-1} of expected biomass), whereas the SM+ treatment received only half dose of N (60 kg ha^{-1}). The culture grew under rainfed conditions; a total of 196 mm of rain fell during the crop cycle. Sorghum was harvested at flowering stage (August, 30 2014). The SM+ and SM- forages were ensiled in bales wrapped in polyethylene film appropriately marked for plot of origin.

3.4.1a Feeding trial

Twenty-eight lactating dairy cows (Italian Friesian cattle) were divided into SM- and SM+ groups (parity 1.83±2.09 vs 1.85±2.17, days in milk 140±60.2 vs 143±60.1 d, milk production 26.8±4.5 vs. 27.3 ±6.1 kg/head/d) fed the same TMR differing only in respect to the presence of SM- or SM+ sorghum silage (Table 9a). Animal handling and milk sampling were alike as the maize trial.

3.5a Sampling and Analyses

Before seeding, soils from each plot (i.e. MM+, MM-, SM+, SM-) were sampled by collecting and mixing five sub-samples from the topsoil (0–20 cm). Soil samples were air-dried at room temperature and sieved through a 2-mm nylon sieve to remove coarse debris. Chemical and physical properties were determined according standard methods (Violante, 2000) and are shown in Table 3a.

Percentages of colonization were measured 30 d after seeding by collecting 3 samples, each consisting of 3 roots, from each plot. Roots were washed out from the soil and their internal mycorrhizal hyphae stained by the method of Phillips and Hayman (1970).

Percentage of colonization (the percentage root length with internal hyphae of arbuscular mycorrhizal fungi) was measured by the gridline intersect method (Giovannetti and Mosse, 1980).

Yield components and plant traits were estimated at harvesting. Yield components were determined as follows. For each plot, the plants from 3 rows 10 m long were manually cut at 10 cm from ground and weighted to determine biomass yield. Then plants were separately chopped per row so that four samples/plot were obtained. Samples were oven-dried (65°C until constant weight) to determine dry matter (DM). Total N and crude protein (CP) were determined by the Kjeldahl procedure (Association of Official Analytical Chemistry, 1995). Net energy for lactation was calculated according Andrieu and Demarquilly (1987) and expressed as Unité Fourragère Lait (UFL) (1 UFL=1700 kcal of net energy for lactation) (Vermorel, 1988).

Plant traits was evaluated on 4 samples/plot, each consisting of 3 plants. The following parameters were recorded: plant height, green and dead leaves, culm number (only for sorghum plants), area of green leaves by Leica Quantimet 500 image analyzer (only for maize plants). Plants were separated into culms, leaves, cobs and panicles and DM determined.

The silages were sampled by a core sampler. The upper, central and lower part of each maize silobag was sampled (3 subsamples/part) to have 3 samples/plot. For sorghum, 3 bales for each plot were sampled (three subsamples per bale). pH of silages was measured by a portable Crison 507 pH-meter. In laboratory, the ensiled forages were analyzed for DM, ash (Association of Official Analytical Chemists, 2002) soluble protein and non-protein nitrogen (NPN) (Licitra et al., 1996), neutral detergent fiber (NDF) and acid detergent fiber (ADF), acid detergent lignin (ADL) (Van Soest et al., 1991). Starch was determined by using a Polax-2l polarimeter in 200 mm long observation tubes.

During the feeding trial, milk from each cow were collected in sterile plain jars, kept refrigerated at 4°C and sent to the laboratory for milk composition analyses (Milkoscan 203, Foss Electric FT 6000, Foss Electric) to be conducted the same day of collection. To determine fatty acid composition and volatile compounds milk from each group was pooled and sampled. Six samples (200 ml each) were taken and stored at -4 °C pending analysis. Three samples were used to determine milk FA composition after lipid extraction (Hara and Radin, 1978) and the trans esterification of triglycerides into the fatty acid methyl esters (FAME), according to the procedure described in details in chapter 3. Milk volatile compound were determined on three samples, on alternate-day sampling. Volatile compounds were extracted according to Villeneuve et al. (2013). The fractions obtained were

analyzed by GC/MS Hewlett-Packard 6890N (Agilent Technologies, Palo Alto, CA, USA) equipped with a capillary column &W HP-5MS (30 m x 0,25 mm i.d .x 0,25 μm Film Thickness; J&W Scientific, Folsom, CA, USA). The details of procedure is reported in chapter 2.

3.6a Statistic

Data were analysed using the SAS statistical package (SAS Institute, Cary, North Carolina, USA). The maize and sorghum trials were analyzed separately. The effect mycorrhizal treatment on crop yields and plant traits was assessed using a completely randomized design with subsampling. Significance among means was compared using the least significance differences test (lsd, $\alpha = 0.05$). The plot was the experimental unit. One-way analysis of variance per repeated measures (proc. Mixed) was used to test the effect of diet containing mycorrhizal or non mycorrhizal forage on milk traits. The cow was the experimental unit with the sampling day as the repeated measure. Milk FA and volatile compounds were analyzed by one-way analysis of variance (t test <0.05). Tendencies to differences were accepted if $0.05 < P < 0.10$.

4a. RESULTS

4.1a Forage Production and Quality

4.1.1a Maize

Although the growing season of maize was less hot and rainiest that normal (Table 2a), water deficit conditions occurred at the water turn of maize tasselling (VT) and silking (R1) stage.

Table 3a shows the characteristics of soil in farm A and B. The data for farm A indicated that the experimental soil was clay (clay content is 65% of fine particles), with pH (8.2) falling in the range of alkalinity (i.e. pH in water 8.2÷8.6), had a good availability of total carbonates (30 g/kg di total $CaCO_3$), organic C (21 g/kg) and organic matter (36 g/kg). The value of C to N ratio (9.2) indicates that organic matter was well humified. Also the N (2.3 g/kg di N_{tot}) and P (68.7 mg/kg di P_2O_5) availability was rather good. The clay nature of the soil, combined with the presence of an adequate level of humified organic matter, ensure high capacity of cationic exchange (CSC> 20 meq/100 g of soil) and a high degree of saturation in bases (GSB> 85%). Calcium was the most abundant cation (77%), followed by K (3.4%) and Na (0.78%).

Table 4a shows the rates of mycorrhizal colonization, the yield components and the plant traits of mycorrhizal (MM+) and non-mycorrhizal maize (MM-). The MM+ had higher percentages of root infection ($\alpha<0.05$), with values above 25%, i.e. the threshold to validate effectiveness of inoculation process.

Maize plant traits partly differed between treatments. The culm weight, the number of green leaves and, consequently, the leaf area were significantly higher ($\alpha<0.05$) in MM+.

As results of these differences, mycorrhizal maize had higher ($\alpha<0.05$) yields of biomass, DM and UFL and a tendency ($\alpha<0.10$) of CP yield ha^{-1} to be higher.

Table 6a reports chemical composition of MM+ and MM- maize silages. No differences were observed for any of parameters examined ($\alpha>0.05$).

4.1.2a Sorghum

In similarity to the maize trial, the growing season of sorghum crop was less hot and rainiest that normal (Table 2a). The experimental soil was clay/sandy in texture, alkaline (pH 8.3) and had a good availability of total carbonates (70.7 g/kg $CaCO_3$). In contrast, with the soil in farm A, the B soil was poor of organic C (6.0 g/kg) and organic matter (10 g/kg). The low value of the C to N ratio (i.e. 5) indicates a limited humification of organic matter. The

availability of N (1,2 g/kg total N) and P (3.3 mg/kg di P_2O_5) was also poor, and the CSC was low. Magnesium was the most abundant cation (58.7%), followed by Ca (25.4%), Na (9.5%) and K (3.9%) (Table 3a).

The mycorrhizal treatment of sorghum seeds was effective as evidenced by the statistically higher rate of infection ($\alpha<0.05$) (Table 5a). In regards to plant traits, statistically higher values ($\alpha<0.05$) were observed in SM+ for leaf and culm weights. Moreover, statistically higher ($\alpha<0.05$) values of DM, UFL and CP yields were observed for SM+, except biomass yield ($\alpha<0.10$) (Table 5a).

In contrast with results observed for maize silage, the quality parameters differed between SM+ and SM-, except starch and NPN, for which tendencies for statistical significance ($\alpha \leq 0.10$) were observed (Table 7a), and DM ($\alpha>0.05$).

4.2a Milk Yield, Milk Quality

4.2.1a Maize

Table 10a presents DM intake, milk traits of buffalo cows fed diet containing MM+ and MM- maize silage.

DM intake was very close in MM+ and MM- groups (respectively, 16.0 kg vs 16.1 kg/head/d). In similarity, also the average milk production was almost similar ($P>0.05$) in the two groups, as well as the fat, protein and lactose contents.

Concerning fatty acid composition, slight and insignificant ($P>0.05$) differences were observed between MM+ and MM- milk (Table 13a).

In Table 14a are presented the volatile compounds identified in milk of animals fed mycorrhizal maize and sorghum. Data are expressed as a percentage change from control milk (i.e. M- diet). The MM+ milk had a significantly higher concentration of acids, ketones, aldehydes and compounds such as dimethyl sulfide, ethyl benzene and p-xylene. On the contrary, the esters were present in greater quantities in the MM- diet.

4.2.2a Sorghum

DM intake of mycorrhizal and non- mycorrhizal sorghum was, respectively for SM+ and SM-, 21.0 vs 20.7 kg/head/d (Table 11a). Milk yields, milk fat, milk protein and milk fatty acid composition were not affected by the use of the mycorrhizal forage ($P>0.05$) (Tables 11a and 13a).

Similarly to what highlighted for buffaloes, milk from cows fed the mycorrhizal diet has a higher concentration of acids, ketones, aldehydes. Even here, the esters followed a reverse trend, being more abundant in the mycorrhizal diet (Table 14a).

5a. DISCUSSION

Although the soils of the two experimental sites were both alkaline, they differ substantially in terms of fertility. The high availability of N and P, and the presence of well-humified organic matter allow defining fertile soil in farm A. On the other hand, the low nutrient availability and the limited presence of humified organic indicate that the fertility of soil B was, in whole, rather limited.

The low supplies of fertilizers (halving the dose of N and elimination of P) associated with the use of mycorrhizal fungi, did not determined any yield reduction, but improves several yield traits. These results are consistent with the fact that many plant traits of both maize and sorghum plants were positively influenced by the mycorrhizal treatment. These results are of particular interest for sorghum crop, since the soil in farm B had a low availability of N and, particularly, of P. Furthermore, since sorghum was cultivated under rainfed condition, it is possible to exclude nutrient inputs through the water of irrigation.

An increment of DM yield was observed by Sabia et al. (2015) on mycorrhizal maize cultivated under low inputs. The meta-analysis carried out by Lekberg and Koide (2005) evidenced that mycorrhizal colonization can increased overall yield in field, irrespective of plant type and management practice (on average 23% ±8). The basis of these improvements is probably due, among the functions explicated by AM fungi towards higher plants, to the exchange of nutrients (Selosse et al., 2004). Due to extra-roots mycelium, the AMF increase the assimilation of nutrients with low mobility or present in small concentrations (Miransari et al., 2001; Finlay, 2008; Daei et al., 2009).

Significant differences between chemical composition of mycorrhizal and not mycorrhizal silages were observed for sorghum which resulted in less fiber and more protein. These results, together with the data observed for yield, indicate that mycorrhizal had a greater accumulation of DM and nutrients, without a deterioration in quality of the fiber. Other authors (Cazzato et al., 2012; Sabia et al., 2015, Sabia et al., 2015b) found an increment of DM yield, and CP percentage without any increment of fiber. Furthermore, similarly to what we observed, Cazzato et al. (2012) detected on mycorrhizal triticale a different distribution of protein fractions in the plant. In general, fodder quality tends to decline as DM yield improves (Sukhchain and Sidhu, 1992). The higher protein content of mycorrhizal sorghum may be related to an higher uptake of N from the soil (Mishra et al., 2008). Cazzato et al. (2012) suggest that the use of inoculation could modify the distribution of protein fractions in the plant.

The lack of consistency between the sorghum and maize trial for forage quality may be due, beside differences in pedological conditions and agronomic techniques, to the stage of maize cutting, that, as previous reported, was at wax stage. Lestingi et al. (2007) reported that the positive effect of bio-activators, including spores of mycorrhizal fungi belonging to the specie Glomus, is more evident during the first phenological stages. The advanced vegetative stage of maize at harvesting is also evident by the fact that the contents of NDF and starch of the two silage MM+ and MM- were higher compared to the values listed by Mazzinelli et al. (2015) for the same hybrid Indigo 600.

In regard to feeding trials, there were no problems of palatability of both rations. The observed DM intake values were is in line with the typical data of lactating cows and buffaloes at similar stages of lactation. No effect of mycorrhizal treatment were observed on milk yield and quality and on fatty acid profile of both buffaloes and cows. In the literature there are no studies examining the influence of mycorrhizal fodder on milk production and this is the first report attesting the lack of effect of mycorrhizal inoculation on animal performances.

The effect of mycorrhizal inoculation on milk volatile compounds was the same for both species of animal (buffaloes and cattle) and forages (maize and sorghum). Mycorrhizal milk have smells that may remind gorgonzola and smoked cheeses, and also herbal notes and citrus. Several papers have investigated secondary compound patterns of mycorrhizal roots such terpenoids (Akiyama and Hayashi, 2002) and flavonoids (Larose et al., 2002). In addition, indirect evidence suggests that AM fungi can also affect the volatile compounds produced in the leaves (Copetta et al., 2006). Sun and Tang (2013) reported an increased presence of acids in mycorrhizal sorghum plants. Nevertheless, also in this case, no studies examined the influence of mycorrhizal forages on milk volatile compounds.

6a. CONCLUSIONS

The results of this research indicate that, for both forage crops studied:

- no reductions of yields were found associating a diminished supply of fertilizers, to the use of mycorrhizal fungi;

- the forage produced by using mycorrhizal treatment may have better chemical characteristic if the forage is harvested at right phenological stage

- there are no significant effects on quantity and quality of the milk produced by both buffaloes and cows.

Therefore, it can be concluded that the use of mycorrhizal fungi has a sure advantage from the environmental point of view, which may, ultimately, be transformed into economic advantage when rations largely based on mycorrhizal fodder are used.

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TABLES

Table 1a. Characteristics of Farm A and B

	Farm A	Farm B
Localization	Sele plain, Salerno province	Fortore mountains, Benevento province
Climate	Csa sensu Koppen	Csb sensu Koppen
Specie raised	Bubalus bubalis	Bos Taurus
Breed	Mediterranean Italian buffaloes	Italian Friesian, Italian Red Pied cattle
Usable agricultural area, ha	154	58.5
Forage crops	Maize, Grass mixture, Alfalfa, Sorghum (Bicolor x Sudan)	Barley, Alfalfa, Sorghum (Sudan x Sudan), Triticale, White Clover
Available grazing areas, ha	-	-
Irrigation water	Yes	No
Lactating Cows, n	368	60
Dairy product	Mozzarella buffalo Campana PDO cheese	Caciocavallo di Castelfranco, TFP cheese. Fresh milk
Feeding	Total mixed ration	Total mixed ration

Table 2a. Climatic condition during the 2014 growing season

	Farm A				Farm B			
	Temperature, °C		Precipitation, mm		Temperature, °C		Precipitation, mm	
Month	Mean	Deviation[1]	Total	Deviation[1]	Mean	Deviation[1]	Total	Deviation[1]
June	21.7	-0.7	37.4	16.1	18.4	0.0	104.0	43.1
July	22.8	-2.6	78.8	63.4	19.4	-2.0	50.8	13.7
August	24.1	-1.2	4.40	-5.9	20.7	-0,8	41.4	15.4
September	21.9	0.0	141.3	57.9	16.9	-0,5	46.0	-26.2

[1]Deviation: actual minus 10-yr monthly average.

Table 3a. Selected physical and chemical properties (mean ± sd) of soils of farm A and B

	FARM A	**FARM B**
Texture	Clay	Clayey Sandy/Clay Loamy-Sandy Clay
Sand (g/kg)	170±69	574±191
Silt (g/kg)	184±12	213±119
Clay (g/kg)	646±68	213±72
pH H_2O	8.2±0.1	8.3±0.3
Electrical conductivity extract 1:5 (ds/m)	0.1±0.0	0.9±1.1
$CaCO_3$ (g/kg)	30.0±5.6	70.7±31.5
N total (g/kg)	2.3±0.7	1.2±0.1
Available P (Olsen) (mg/kg)	30.0±9.4	3.3±0.5
Organic C (g/kg)	21.1±7.5	6.0±0.8
Organic matter (g/kg)	36.0±1.3	10.0±0.1
Organic C/organic N	9.2±0.7	5.0±0.3
Cation exchange capacity (cmol(+) kg-1)	38.5±1.8	12.6±3.4
Exchangeable Ca (cmol(+) kg-1)	29.5±1.2	3.2±3.5
Exchangeable Mg (cmol(+) kg-1)	7.4±1.0	7.4±1.4
Exchangeable K (cmol(+) kg-1)	1.3±0.6	0.5±0.1
Exchangeable Na (cmol(+) kg-1)	0.3±0.1	1.2±1.0
Available Mn ass. (mg/kg)	13.6±0.4	5.2±1.2
Available Cu (mg/kg)	5.7±0.4	1.1±0.6
Available Fe (mg/kg)	21.4±4.1	8.6±1.1
Available Zn (mg/kg)	0.8±0.3	0.6±0.1

Table 4a. Rate of infection, plant and yield traits of mycorrhizal (MM+) and not mycorrhizal (MM-) maize

	MM+	MM-	SEM	LSD
Percentage of infection, %	48.2	19.1	0.18	1.1
Leaf Area, cm^2	6769	6238	35262	466
Principal culm height, cm	314	299	72.10	-
Green leaves $plant^{-1}$, n	13.7	12.5	0.21	1.1
Leaf weight $plant^{-1}$, g DM	60.0	50.8	35.3	-
Culm weight $plant^{-1}$, g DM	125.5	92.0	142	29.6
Cob/ weight $plant^{-1}$, g DM	275.7	233.8	130	28.3
Biomass yield, Mg ha^{-1}	76.3	65.7	23.9	3.8
Dry matter yield, Mg ha^{-1}	27.5	23.0	3	1.3
Crude protein yield, Mg ha^{-1}	0.18	0.17	0.001	-
UFL yield ha^{-1}	25031	20909	340867	1450

LSD, $\alpha = 0.05$

Table 5a. Rate of infection, plant and yield traits of mycorrhizal (SM+) and not mycorrhizal (SM-) sorghum

	SM+	SM-	SEM	LSD
Percentage of infection, %	43.5	19.9	0.6	4.8
Principal culm height, cm	178	176	625	/
Green leaves $plant^{-1}$, n	7.0	6.6	0.23	/
Leaf weight $plant^{-1}$, g DM	3.6	2.8	0.50	/
Culm weight $plant^{-1}$, g DM	17.6	13.2	1.9	3.0
Cob weight $plant^{-1}$, g DM	4.6	3.9	0.9	/
Biomass yield, Mg ha^{-1}	46.3	36.1	714	/
Dry matter yield, Mg ha^{-1}	14.0	10.1	21	3.2
Crude protein yield, Mg ha^{-1}	0.13	0.9	0.001	2.5
UFL yield ha^{-1}	8371	6459	775679	1895

LSD, $\alpha = 0.05$

Table 6a. Quality of mycorrhizal (MM+) and not mycorrhizal (MM-) maize silage

	MM+	MM-	SEM	LSD
Ash, % DM	5.4	5.6	0.28	/
Crude protein, %DM	7.3	7.9	0.16	/
NDF, % DM	40.6	41.8	5.28	/
ADF, % DM	22.6	21.1	0.84	/
ADL, % DM	1.4	0.9	0.01	0.38
Soluble Protein, % DM	2.4	2.8	0.18	/
NPN, % DM	0.6	0.8	0.01	/
Starch, % DM	40.3	38.7	0.91	/

LSD, $\alpha = 0.05$

Table 7a. Quality of mycorrhizal (SM+) and not mycorrhizal (SM-) sorghum haylage

	SM+	SM-	SEM	LSD
Ash, % DM	9.05	8.11	0.55	/
Crude protein, %DM	11.7	9.7	0.64	1.84
NDF, % DM	64.3	62.2	0.18	0.97
ADF, % DM	42.0	39.8	0.15	0.91
ADL, % DM	5.9	4.5	0.05	0.52
Soluble Protein, % DM	7.7	5.9	0.33	1.32
NPN, % DM	0.1	0.9	0.009	/
Starch, % DM	3.0	2.7	0.004	0.15

LSD, $\alpha = 0.05$

Table 8a. Composition and chemical characteristics of diet fed to MM+ and MM- lactating buffalos in farm A

	MM-	**MM+**
Feedstuffs		
Mycorrhizal Maize silage	-	20.0
Not mycorrhizal Maize silage	20.0	-
Alfalfa hay	2.0	2.0
Meadow hay	2.5	2.5
Mash maize	3.0	3.0
Maize flour	1.5	1.5
Concentrate*	3.0	3.0
Chemical composition		
Crude protein, %DM	14.0	14.1
NDF, %DM	37.3	38.0
ADF, %DM	24.7	25.4
UFL, kg/DM	0.87	0.87

**Based on soybean meal (70%) sunflower meal (20%) and barley meal (10%)*

Table 9a. Characteristics of diet fed to SM+ and SM- lactating cows in farm B

	SM-	**SM+**
Feedstuffs		
Mycorrhizal sorghum haylage	-	22.0
Not mycorrhizal sorghum haylage	22.0	-
Meadow hay	6.0	6.0
Commercial concentrate	14.0	14.0
Water	4.0	4.0
Chemical composition		
CP, %DM	15.7	16.2
NDF, %DM	42.5	42.0
ADF, %DM	26.4	26.0
UFL, kg/DM	0.85	0.85

Table 10a. Intake and milk yield of buffalo cows fed MM+ and MM- diet

	MM+	MM-	SEM	P
Ingestion of dry matter, kg/d	16.0	16.12	0.12	NS
Milk production (kg/d)	11.1	11.3	0.93	NS
Fat (%)	9.1	9.0	0.36	NS
Protein (%)	4.5	4.5	0.09	NS
Lactose (%)	4.9	4.9	0.06	NS

Table 11a. Intake and milk yield of dairy cows fed SM+ and SM- diet

	SM+	SM-	SEM	P
Ingestion of dry matter, kg/d	21.0	20.7	0.42	NS
Milk production (kg/d)	26.8	27.3	0.93	NS
Fat (%)	3.6	3.8	0.12	NS
Protein (%)	3.4	3.3	0.08	NS
Lactose (%)	4.8	4.8	0.08	NS

LSD, $\alpha = 0.05$

Table 12a. Fatty acid composition of buffalo milk from MM+ and MM- diet

Fatty acid	MM+	MM-	SEM	P	Significance
C4:0	7.4	9	0.46	0.13	NS
C6:0	3.64	4.76	0.31	0.12	NS
C8:0	1.9	2.21	0.01	0.44	NS
C10:0	3.15	3.91	0.23	0.14	NS
C12:0	3.74	4.37	0.003	0.18	NS
C14:0	14.11	14.77	0.20	0.15	NS
C141:0	1.60	1.70	0.09	0.55	NS
C16:0	39.43	39.71	0.91	0.36	NS
C16:1	2.73	2.76	0.26	0.94	NS
C18:0	5.92	4.72	0.61	0.30	NS
C18:1 n-9t	0.25	0.38	0.13	0.57	NS
TVA	0.15	0.12	0.01	0.29	NS
C18:1 n-9c	11.22	9.29	0.94	0.28	NS
C18:2 n-6c	1.10	0.89	0.10	0.28	NS
C18:2 n-6t	0.20	0.14	0.01	0.06	NS
C18:3 n-3	0.13	0.10	0.007	0.08	NS
CLA	0.30	0.25	0.03	0.32	NS
Others	2.97	2.67	0.09	0.16	NS
UFA	17.71	15.65	0.72	0.18	NS
SFA	79.31	81.68	0.73	0.14	NS

Table 13a. Fatty acid composition of cow milk from SM+ and SM- diet

Fatty acid	SM+	SM-	SEM	P	Significance
C4:0	5.53	7.69	0.60	0.11	NS
C6:0	3.40	4..35	0.20	0.06	NS
C8:0	1.95	2.16	0.13	0.38	NS
C10:0	4.24	4.17	0.37	0.90	NS
C12:0	4.79	4.64	0.38	0.81	NS
C14:0	14.10	14.52	0.19	0.26	NS
C141:0	1.93	1.77	0.07	0.27	NS
C16:0	37.94	38.78	0.58	0.43	NS
C16:1	1.87	2.34	0.28	0.37	NS
C18:0	5.82	4.86	0.47	0.29	NS
C18:1 n-9t	0.93	0.65	0.17	0.40	NS
TVA	0.16	0.15	0.01	0.75	NS
C18:1 n-9c	11.66	9.31	0.71	0.12	NS
C18:2 n-6c	1.73	1.17	0.16	0.11	NS
C18:2 n-6t	0.26	0.19	0.04	0.35	NS
C18:3 n-3	0.34	0.23	0.06	0.37	NS
CLA	0.40	0.28	0.04	0.18	NS
Others	2.89	2.71	0.11	0.40	NS
UFA	19.31	16.11	0.995	0.12	NS
SFA	77.79	81.17	0.89	0.09	NS

Table 14a. Volatile compounds of milk from maize and sorghum (M+ and M-) diets (data are expressed as a percentage change from M- milk)

Compounds	Maize MM-/MM+ (%)	Sorghum SM-/SM+ (%)
Ketones		
Acetone	+114*	-2.08
2-butanone	-15	21***
2-heptanone	-57**	
2-nonanone	-33**	-47*
Acids		
Butanoic acid	-21*	-74***
Hexanoic acid	-4	-51*
Octanoic acid	-33*	-52
Nonanoic acid		-100***
Decanoic acid	-22*	-48***
Undecanoic acid	4	-44***
Tetradecanoic acid	1	-27
Esters		
Ethyl acetate	+***	+***
Alcohols		
2-ethyl -1-hexanol (t)	-7	2
Aldehydes		
Hexanal	-6	36
Nonanal	-47**	8
Decanal	-100***	-22
Others		
Dimethyl sulfide (t)	-100***	
Ethyl benzene (t)	-92***	
p-xylene (t)	-83***	

CHAPTER 2

EFFECT OF DIFFERENT FORAGE PRESERVATION METHODS ON CHEESE QUALITY

In Italy the dairy sector is of great importance and more than 70% of the milk production is used in the manufacture of cheese (Istituto Nazionale di Statistica, 2005). A wide range of dairy products is available, characterized by distinctive organoleptic proprieties achievable only within specific geographical areas. A great part of these dairy products have some EU' labels or are included in the Italian "Traditional Food Product" (TFP) list.

The EU' labels (i.e., **PDO**, Protected Designation of Origin; **PGI**, Protected Geographical Indication; **TSG**, (Traditional Specialty Guaranteed) have been introduced to encourage diverse agricultural production and protect product names from misuse. These labels link the products to their geographic origin and to a specific production process (mainly traditional). In particular, the PDO mark is assigned to agricultural products and foods produced, processed and prepared in a given geographical area using recognized know-how. One of the most representative Italian PDO cheese is *Mozzarella di Bufala Campana* (MBC), a fresh cheese with high moisture and fat content, characterized by a soft body juicy appearance and by a pleasant, fresh, sour and slightly nutty flavor (Devirgiliis et al., 2008). MBC is exclusively manufactured in specific areas of central and southern Italy and it is made with milk from locally raised water buffalos. MBC received the European Certification PDO (Product of Designated Origin, EEC Regulation no. 1107) in June 1996, later modified with the extension of the geographical area (N. CE: IT/PDO/117/0014/20.09.2002) and it is bound to a specific production protocol (Coppola et al., 1988).

TFP are some typical local products, not yet identified as PDO, defined as *Agrifood products whose methods of processing, storage and ripening are consolidated with time according to uniform and constant local use* (Ministero Agricoltura, 1999). TFP provide an ample variety in food choice and are often recognized by consumers with characteristics linked to regional identity (Guerrero et al., 2009). TFP also constitute an important element of European culture, identity, and heritage (Committee of the Regions, 1996; Ilbery B., and Kneafsey M., 1999) contributing to the development and sustainability of rural areas, protecting them from depopulation, entailing substantial product differentiation potential for producers and processors (Avermaete et al., 2004). The *Caciocavallo di Castelfranco* is

a TFP cheese produced in a specific mountain area between Apulia and Campania regions in southern Italy. This caciocavallo is made by using milk from locally dairy cows. Beside concentrates, animals are fed pasture and other local produced forages. The characteristics of *Caciocavallo di Castelfranco* production are the use of natural whey culture and liquid calf rennet, the manual stretching of the curd in hot water, the shaping into cheese with no pressing, and the salting in brine. The cheese has a typical flask-like shape with a short neck and a small round top, it has a medium hard texture and yellow color as affected by season and ageing time. It can be consumed as semi-ripened (aged from 2 to 4 months) or ripened cheese (more than 4 months).

AIMS

In the face of strong national and international competitors, small dairy producers have to focus on product' quality in order to favor the formation of market niches. In the food production chain the feeding regimen plays a central role in defining the relationship between typical products and the place of origin (Grappin and Coulon, 1996). It is known that different forage in the cow's ration can affect some milk and cheese characteristics e.g. microbiological profile, chemical composition, sensorial attributes and consumer liking (Kalač, 2011).

In the light of these considerations, in this research line examined the influence of forage preservation method on quality of *Caciocavallo di Castelfranco* TFP (experiment b), and *Mozzarella di bufala campana* PDO (experiment c).

EXPERIMENT B

EFFECTS OF SUBSTITUTION OF SILAGE WITH HAY ON CACIOCAVALLO CHEESE QUALITY

1.b INTRODUCTION AND AIMS

Hay-making is a preservation method highly weather dependent. As a consequence, is not easy to produce good quality hays in dairy farms of internal mountains areas of southern Italy, where delays in the cutting of forage due to unfavorable weather condition are quite common.

Ensiling is a preservation method that can be used with almost any plant material, it is a less weather dependent process. In areas with wet and variable weather during forage harvesting, silage making largely replaced haymaking (Weinberg and Ashbell, 2003).

Silage can be defined as any plant material that has undergone by natural or artificial acidification by fermentation with the anaerobe bacteria (McDonald, 1981). Actually, silage making is an essential annual operation in many ruminant farms of the large plains. Compared to hays, silages present several advantages since provide a long term stored forage with high quality, can increase crop productivity and can reduce feeding costs. In addition, there are wider crop options and minor harvest losses. On the other hand, there are also some potential negative features of silage including its bulkiness in handling and storage, the necessity of additional equipment and structures for harvesting, storing, and feeding, the complete loss if not stored properly, the being not readily marketable off-farm, and the job for feeding soon after removal from the silo to minimize spoilage (McDonald, 1981). However to use of a diet with higher intake of hay may affect animal performance (Leto et al., 2002). Silages offer economic and technical benefits, and they can influence milk and cheese quality. The quality of silage has great place to define the effects on the cheese and milk products. If silage is poorly preserved, the presence of butyric spores in silage and in milk may create serious defects, particularly for the ripened cheese, such as late swelling and unpleasant taste and odor (Pa, 2011).

Some authors observed an influence of silages also on the technological characteristics of milk (Coulon et al., 2004; Macheboeuf et al., 1993; Verdier-Metz et al., 1998). Cheese is an important milk product source of the fat soluble vitamins and tocopherols in the human diet. It was found that silage milk has less content of both retinol and tocopherols than hay milk. However, silage is a richer source of provitamins because of the higher loose of this compounds during haymaking. Furthermore, regarding to the

water-soluble vitamins, the only difference was observed in the riboflavin concentration that was higher in silage than hay (Kalač, 2011).

Several studies examines the effects of conservation method on sensorial properties and volatile compounds. The most important effect of silage feeding is on the sensorial properties linked with consumer preference. Some authors (Agabriel et al., 1999; Verdier-Metz et al., 1998; Sheath et al., 2001) have found that cheese made from silage milk were yellower, and slightly more bitter than those made from hay milk. It has been observed that cheese was made with milk from without silage fed was preferred to others (Stefanon and Procida, 2004). The compounds that influence sensorial properties come from grass, from preservation operations (Kalač, 2011), from inhaled air, from the digestive tract (Urbach, 1990 ; Desagé et al. 1996), from microbiota activity in fresh milk (Martin et al. 2001). Terpens are responsible of specific flavors, and in dairy products are mainly related with feeding. Terpens content in forages is affected mainly by botanical composition and stage of maturity. Some terpen components were observed higher in the hay based diet than silage (Toso et al., 2002).

Therefore, although the utilization of silages as feed for dairy cow is widespread, remains perplexity about the effects of silages on cheese quality (chemical, physical, microbiological and technological characteristics). It is well known that animal feeding can influence quality of dairy products within the same cheese-making chain and, in light of this consideration, this study aimed to examine the influence of use of silage on the TFP Caciocavallo di Castelfranco cheese.

2 b. MATERIAL AND METHOD

2.1b Feed Production

The experiment was carried out in a dairy cattle farm (usable agricultural area about 50 ha) located in the Fortore Mountains, Campania region, southern Italy. The farm raised 70 Italian Friesian cows and produced *Caciocavallo di Castelfranco* cheese exclusively from milk produced on farm. Other farm characteristics are shown in Table 1a.

On May 2014, a 7-ha dryland field (690 m a.sl) was sown with *Sorghum sudanese* by using the hybrid Sudangrass x Sudangrass Hermes (Alforex®) suitable for both ensiling and haymaking. At harvesting time, four months later, forage from 3.5 ha was cut, dried in the field for 3 days and preserved in bales (400 kg). Forage from the other 3.5 ha was cut by using a tractor-mounted mower-conditioner and immediately ensiled in plastic bags (700 kg) without any additives. The bales and the bags were stored in covered barns until the cheese-making trial started (March 2015).

2.2b Experimental Design, Cheese Making and Sampling Procedure

Forty-four lactating Holstein–Friesian cows were equally divided into SILAGE and Hay group, balanced for days in milk (169±116 vs. 165±109 d, respectively for the silage and hay groups), milk yield (23.4±8.2 vs. 23.7±7.4 kg/head/d), milk fat (4.4±0.8vs. 4.5±0.9%) and milk protein (3.4 ± 0.5 vs. 3.6 ± 0.4). Cows were fed once daily (15:00 h) in amount to provide approximately 10% orts for ad libitum consumption. The HAY group was fed a total mixed ration based on sorghum hay that, in the SILAGE group, was substituted by sorghum silage. A small amount of sorghum hay (6 kg as fed) was included in the SILAGE diet in order to provide a basic level of physically effective fiber to reduce the risk ruminal acidosis. The diet composition is in Table 1b. The ration was re-approached several times daily to ensure unlimited access to feed. Cows were housed in two adjacent free stall pens with concrete floor and equipped with feed manger, drinker, and covered paddock and handled in similar way in terms of feeding and management. The milking were done twice daily (05:00 and 17:00).

After 10 days of adaptation to diet, in 3 consecutive days (d1, d2, d3), bulk milk of each feeding group was separately collected (on average 200 kg) and caciocavallo cheese was manufactured according to the procedure presented in the Figure 1b.

The HAY and SILAGE cheeses were made at the same time in separate vats. Each vat was filled with milk and inoculated using 2 separate natural whey culture from the previous day's manufacture as starter. An additional cheese making was performed on the

day before the start of the trial, to produce 2 separate natural whey cultures (HAY and SILAGE) to be used as starter in the first cheese making day. For each experimental day, 8 pieces (2.5 kg) of cheeses were produced.

The cheeses remained in the farm until the end of the predetermined periods of ageing, i.e. 0 (after salting), 30, 60, and 90 d, and then were sent to the laboratory.

For each day of cheese making, bulk milk of each group were sampled, packed in multiple 200-mL plastic flasks and, under refrigeration, sent to the laboratory. Moreover, feed intake of the two groups was measured by the difference between feed administered and orts. Samples of each rations were collected separately for each day, dried in oven (65ºC) with forced ventilation to measure dry matter content. Subsequently, samples were unified per diet, grinded in a mill equipped with a wire mesh of 1 mm, and stored until analysis.

2.3b Analysis

2.3.1b Chemical analysis of feed, milk and cheese

Feed samples were analyzed for DM, ash, crude protein (Association of Official Analytical Chemists, 2002), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) (Van Soest et al., 1991). UFL was calculated from the chemical composition of feedstuffs.

Milk fat, protein and lactose were determined using a Milkoscan 605 (Foss Electric, Denmark).

Cheese samples were separately analyzed per diet (Hay and Silage), day of production (d1, d2 d3) and ripening time (30, 60, 90 d).

For each sample, about 200 g of cheese were taken at 2 cm from the rind and used to determine chemical composition and to extract fat for fatty acids profile. Crude protein was determined by the macroKjeldahl method, moisture content was determined by oven drying and crude fat by the method of Folch *et al.* (1957), followed by a purification phase by using saturated sodium chloride solution.

The extraction of volatile compounds in milk was performed according to Villeneuve *et al.* (2013): 22.5 g of milk was transferred into 50 ml bottle, then was added 30 µl of 2-methyl-3-heptanone as internal standard (408 mg L^{-1}) and 2.75 g of sodium phosphate (NaH_2PO_4). Sample bottle was put in apparatus that can regulate water temperature and stirring of sample. The sample was magnetically stirred for 5 min at 55 °C. The SPME fiber was inserted through the Teflon septum in the bottle and exposed to sample headspace 60 min at 55 °C during stirring.

The obtained fractions were analyzed by GC/MS Hewlett-Packard 6890N (Agilent Technologies, Palo Alto, CA, USA) equipped with a capillary column J&W HP-5MS (30 m x 0,25 mm i.d. x 0,25 µm Film Thickness; J&W Scientific, Folsom, CA, USA). The injection temperature was 250 °C. The carrier gas employed was helium (purity 99.9995%) at a constant flow rate of 0.9 ml/min. The oven temperature program was 2 min at 40 °C, then increasing at 6 °C/min up to 160 °C, and following arrived to 210 °C with an increasing rate of 10 °C/min. The identification of the compounds was carried out by comparing the retention times and the mass spectra obtained by taking as reference the pure compounds in the same conditions. Prior to use, the fiber was conditioned at a temperature of 270 °C about for 1 hour. The MS detector was set with an ionisation potential of 70 eV with a GC–MS interface temperature of 250 °C operating in complete scanning range of *m/z* 29–400. Compounds were identified using the NIST 107 and NIST

21 libraries. Each sample was analyzed in triplicate. The area of each peak were computed by using a ChemStation software (Agilent Technologies, Palo Alto, USA).

2.3.2b Cheese color and texture

Measure of both color and texture were made on 4 cylindrical (diameter, 23 mm; height, 11 mm) samples taken from the upper, central and lower parts of a cheese slice. The mean of all replicates (4 samples*3 areas) was used for statistical analysis.

Color was determined according CIELAB system by the spectrophotometer U-3000 (Hitachi, Tokyo, Japan) driven with a Spectra Magic 1.01 software (Minolta), with the following conditions: D65 as illuminant, and 2° angle observer. Data were reported in the CIE *L*a*b** colorimetric system. The measure of L* (lightness range 0-100) represents black to white, the a* measurements (redness) describe green to red, and the b* measurement (yellowness) represents blue to yellow. Yellow index was calculated according Francis and Clydesdale (1975).

The compression test was performed with an Instron Universal 5565 testing machine (Instron Ltd., High Wycombe, UK). Each sample underwent 2 cycles of 50% compression. The velocity and the load cell of the Instron were 50 mm/min and 500 kg, respectively. The variables determined were hardness (the maximum force of the first compression), cohesiveness (the total energy required for the second compression divided by the total energy required for the first compression), springiness (ability of the sample to recover to its original shape after the deforming force was removed), gumminess (hardness × cohesiveness), and chewiness (springiness × gumminess).

2.3.3b Cheese volatile compounds

The analysis of volatile compounds was carried out for cheese at 30 and 60 d of ripening. Volatile compounds were extracted according to Lee et al. (2003). Briefly; frozen cheese samples were finely grated with a cheese cutter into small pieces about 1 mm in diameter. 25 g of cheese was transferred into 100 ml bottle, previously added with 25 mL of deionised water, 50 µl of 2-methyl -3-heptatone as internal standard (408 mg/ l) and 12.5 g of sodium phosphate (NaH_2PO_4). Sample bottle was put in an apparatus that can regulate water temperature and stirring of sample. The sample bottle was kept for 10 min at 50 °C in the apparatus to melt the cheese. Than sample was magnetically stirred for 20 min at 50 °C. The SPME fiber was inserted through the Teflon septum in the bottle and exposed to sample headspace 30 min at 40 °C during stirring. The condition of analysis and instruments details were similar as previously described for milk.

2.3.4b Sensorial analysis and consumer acceptability

The sensory analysis of caciocavallo cheeses was carried out for cheese at 30, 60 and 90 d of ripening, by using a quantitative–descriptive analysis (QDA) method (Murray et al., 2001). A panel composed of 8 assessors participated to the analysis. All panelists were experienced in the assessment of sensory analysis of similar cheeses. All judges were trained in the assessment of the intensity of sensory stimuli and were involved in the development of the attributes to generate a specific vocabulary for caciocavallo cheese. A single score card with 2 appearance, 6 odor / flavor, 3 taste, and 3 texture descriptors was compiled, representing the consensus profile of sensory characteristics (Table 6b).

In all tests, carried out at about 10.00 a.m., cheese cube samples (1 cm^3) were served in a randomized order. A random 3-digit number was assigned to each sample so that panelists were unable to recognize the treatment. Attributes were evaluated by rating the samples on 100-mm unstructured lines, in details 0-20 weak, 21-40 weak/moderate, 41-60 moderate, 61-80 moderate/strong, 81-100 a very strong. Tests were performed in sensory booths (ISO 8589, 1988) under red fluorescent lights to mask color differences in the samples, except during the evaluation of color, when white fluorescent lighting was used. A slice of apple were provided to neutralize taste between cheese samples. In order to avoid sensory fatigue due to the number of samples, in each session, only three samples were evaluated. The interval between samples was approximately 10 minutes. The panelists evaluated 3 replications (d1, d2 and d3) of each cheese.

Caciocavallo cheeses were also evaluated for consumer acceptability (Kähkönen et al., 1996). A total of 75 consumers (46 females and 29 males) with an age ranging from 26 to 60 years participated in the test. Each participant evaluated six 1 cm^3 cheese cube samples (corresponding to the 3 ripening time x 2 diets) in a controlled sensory analysis laboratory as described for QDA. For each product, consumers expressed an overall liking and a liking according to the following sensory inputs: appearance, taste/flavor and texture. So each consumer expressed 4 preferences. Consumers rated their liking on a 9-point hedonic scale labelled at the left end with “extremely unpleasant” (1), at the right end with “extremely pleasant” (9) and at the central point with “neither pleasant nor unpleasant” (Kähkönen et al., 1996).

2.3.5b Microbiology analysis

In order to determine the evolution of indigenous microbiota during cheese making process and ripening in each day of production the following samples separately per HAY and SILAGES were collected: raw milk, whey culture, inoculated milk, curd at the end of

fermentation process, cheese after brining (0 d) and 30 d and e 60 d. On these samples the following microbial counts were performed according to the procedure described below: mesophilic and thermophilic lactic acid bacteria and streptococci; enterococci, yeasts and molds, *Escherichia coli*. Twenty five grams of solid samples were suspended in 225 mL of sodium citrate (2% w/v) solution and homogenized for 2 min with a stomacher (Lab-Blender 400 Seward Medical, London, UK), whereas, for liquid samples, 10 ml were suspended in 90 mL of sterile quarter-strength Ringer's solution (Oxoid, Basingstoke, UK). Furthermore all the samples were 10-fold diluted with Ringer's solution and inoculated (in duplicate) in appropriate growth media: Plate Count Agar (Oxoid) incubated at 30°C for 48 h to determine total mesophilic bacteria; TBX (Oxoid) incubated at 37°C for 2 h and then at 44°C for 46 h to determine presumptive Escherichia coli; thermophilic and mesophilic cocci Lactic Acid Bacteria (LAB) were counted on M17 agar (Oxoid) with 1% lactose after incubation for 48 h at 42°C and at 30°C, respectively; thermophilic and mesophilic rod LAB were counted on modified(m)MRS agar at pH 6.5 (mMRS: MRS, Oxoid, supplemented with 0.05% L-cysteine/HCl (Sigma-Aldrich, St Louis, MO, USA) and with 0.002% bromophenol blue) prepared according to Lee and Lee (2008) incubated in anaerobiosis (Anaerogen kit, Oxoid) for 48 h at 37°C and at 25°C, respectively; Slanetz & Bartley agar (Oxoid) at 37 °C for 2 h and then at 44°C for 70 h to determine presumptive Enterococci; Dichloran Rose-Bengal Chloramphenicol agar (SIMAD, Italy) at 25°C for 72 h to determine yeasts and molds.

Measurement of pH was performed using a FC2320 pH electrode inserted directly into the samples (Hanna Instruments, Italy).

2.4b Statistic

All statistical analysis were done by using SAS package software.

2.4.1b Chemical composition, texture, color

The effects of the two diets (HAY and SILAGE) and ripening periods (30, 60 and 90 d) were evaluated by analysis of variance per repeated measures (proc. Mixed) considering the fixed effect of Diet (2 level) and Ripening (3 levels) and their interaction (Diet*Ripening). All data are reported as LS means and standard errors of means.

2.4.2b Volatile compounds

The effect of the two diets (HAY and SILAGE) was separately evaluated at 30 and 60 d of ripening by using one-way Anova. Principal component analysis was used to rank the relationship of each variable with diet.

2.4.3b Sensorial analysis and consumer acceptability

Sensory profile data were subjected to ANOVA with assessor (8), replication (3), product (6 = 2 Diet × 3 Ripening time), and their interactions as factors. A further ANOVA was performed using diet (HAY, SILAGE), Ripening time (30-60-90), and their interaction as factors. To identify the most liked product, acceptability data were analyzed by ANOVA, using Diet, Ripening time, and interaction as factors. The relationship between overall liking and attribute liking (appearance, taste/flavor, and texture) was analyzed by linear regression analysis according to the procedure described by (Esposito et al., 2014).

2.4.4b Microbiological analysis

Microbiological results were analyzed by GLM procedure including the effects of Diet (HAY and SILAGE), nature of the sample (L: H, hay; S, silage; M, milk; NWC, natural whey culture; CC, curd immediately after coagulation; CS, curd at end of acidification; C1, C30 and C60, cheese after 1, 30 and 60 days of ripening) and their interaction D*L. The Tukey's test was used to compare the means at $P < 0.05$ significance level. All the values are the mean values (± Standard Deviation, SD) obtained from three independent trials for each trait.

3b. RESULTS

3.1b Chemical composition

The chemical composition of the bulk milk from HAY and SILAGE group is present in the Table 2b. No difference was observed for fat and protein content between two diets ($P>0.05$). Lactose content in HAY milk was significantly higher ($P<0.05$).

The chemical composition of caciocavallo cheese for two diet and the three ripening time are presented in the Table 3b. The interaction of Diet*Ripening did not show any effect. Dry matter content showed an increment from 30 to 90 d as expected ($P<0.05$), but between diets no difference was observed ($P>0.05$). Crude protein content was higher in HAY cheese ($P<0.05$), whereas fat content was not affected neither by Diet nor by Ripening time.

3.2b Color

The color of the caciocavallo cheese as affected by Diet and Ripening time are presented in Table 4b. The interaction Diet*Ripening time was never significant. Except lightness, significant effects ($P<0.05$) of Diet were observed for all parameters, with the SILAGE cheese presenting the higher redness, yellowness and yellow index values.

Lightness, redness and yellow index changed significantly during ripening, but not in liner way. Lightness decreased from 30 to 60 d ($P<0.05$), but no differences were observed between 60 and 90 d ($P>0.05$). By contrast, the a* values were significantly lower only at 90 d ($P<0.05$). The b* values were not affected by Ripening time, and the differences observed for yellow index were due to the changes of the L values.

3.3b Texture

The texture of the caciocavallo cheese as affected by Diet and Ripening time are presented in Table 5b. No significant ($P>0.05$) effects of Diet nor interaction Diet* Ripening were observed. Ripening significantly influenced hardness and chewiness; in particular, hardness were significantly lower at 90 d ($P<0.05$), whereas a linear reduction of chewiness was observed from 30 to 60 to 90 d ($P<0.05$). A similar trend was observed also for springiness, but the differences were below the threshold of significance ($P>0.05$).

3.4b Sensorial analysis

The sensory properties of the caciocavallo cheese as influenced by Diet and Ripening time are presented in Tables 7b and 8b. The sensory attributes of cheese were categorized as appearance, odor/flavor, taste and texture. The appearance attributes (yellow and uniformity) were influenced by Diet, Ripening and Diet*Ripening interaction. HAY cheeses were less yellow than SILAGE ones for any Ripening time except 90 d. The

yellowness of SILAGE cheese didn't change during ripening. On the contrary, the yellowness of HAY cheese showed a significant increment at 90 d of ripening. The differences in uniformity were due more to the interaction than to the single effects of Diet and Ripening. A decrement of uniformity was observed for HAY cheese from 30 to 60 d, whereas the values of SILAGE cheeses changed at the 90 d. Regarding to the flavor/odor attributes, only overall odor and milk were not influenced by Diet and Ripening. The Diet*Ripening interaction was significant only for overall flavor. Grass was influenced only by Diet, with the higher values observed, on average, for SILAGE cheeses. In addition, the overall flavor and butter flavor of SILAGE cheese were higher than the HAY cheese, and they tended to increase during ripening. In addition, the intensity of HAY flavor increased during ripening.

The taste attributes were influenced by Diet, Ripening and, only for bitter, by the Diet*Ripening interaction. SILAGE cheeses were more salty and had more umami taste, with values increasing during ripening. The bitter taste was influenced more by the interaction than the two effects alone. In fact, SILAGE and HAY cheese showed opposite pattern of bitter intensity during the ripening: compared to SILAGE, cheese HAY diet showed a lower intensity of bitter at 30 and 60 d but higher at 90 d.

Regarding the texture attributes, no effects of Diet were observed except for oiliness, whereas Ripening influenced all texture parameters (oiliness, tenderness, elasticity). The interaction of Diet*Ripening was significant only for tenderness. Oiliness was significant higher in SILAGE cheese only at 30 d of ripening. Elasticity decreased significantly from 60 to 90 d for both HAY and SILAGE cheese but no difference was observed between 30 to 60 d. These results are represented graphically in Figures 2b and 3b.

3.5b Consumer liking and slope analysis

Table 9b, shows the hedonic scores for cheese as affected by Diet and Ripening time. All caciocavallo cheese performed well in the consumer tests, as the panelists rated all samples acceptable, with scores >5 (neutral score: neither pleasant nor unpleasant) for overall liking and liking according to appearance, taste/flavor, and texture. Analysis of variance showed that, in terms of overall preference, there are significant differences between the products. The average preference expressed by consumers resulted to be significantly higher for the product HAY ripened for 90 d (6.63) and lower for the product SILAGE ripened 30 d (5.73). All other samples were in a group of intermediate preference. The regression of consumer liking against analytical sensory data can show the most important input driving the acceptance for a specific product (Ward et al. 1999; Moskowitz

and Krieger 1995). Therefore, the relationship between overall liking and liking of specific sensory inputs (appearance, taste/flavor, and texture) was analyzed by linear regression analysis. Data area presented in Table 10b.

Significant differences were observed between a taste/flavor and both texture and appearance ($P<0.05$), whereas no differences were observed between texture and appearance. This result is represented graphically in Figure 4b.

3.6b Microbiological analysis

The mean results of microbial population and the pH values of the HAY and SILAGE samples during cheese making and ripening of Caciocavallo of Castelfranco are reported in Table 11b. The raw milk samples used for cheese making had similar pH values for two dietary groups. At curd acidification pH decreases until the values of 5.11 vs 5.33 for HAY and SILAGE respectively. A 60 d of ripening, ultimate pH slightly increased (5.21 vs 5.39). The raw milk (M) samples had similar mean counts of all microbial groups with a slightly higher number of presumptive mesophilic and thermophilic cocci (Table 11b). Except for enterococci, all the microbial groups were significantly influenced by the type of sample (L) ($P< 0.001$). A significant effect of Diet was observed on the development of mesophilic and thermophilic lactobacilli, mesophilic streptococci and presumptive *E. coli*. Moreover, a significant interaction (Diet *Sample) found on the mesophilic lactobacilli ($P<0.05$).

Yeasts and molds mean values (Table 11b) increased in both HAY and SILAGE groups during the 60 days of ripening and the highest value was observed in the SILAGE cheese samples (4.29 log CFU g^{-1}).

3.7b Volatile compounds

The figure 5b shows PCA of caciocavallo cheese at 30 days of maturation produced with milk from cows fed HAY or SILAGE based diet. The bold characters indicate significant differences between groups.

Ketones and free fatty acids (hexanoic, butanoic, acetic) were higher in SILAGE cheese resulting in a stronger flavor of gorgonzola cheese, caramel and wine vinegar.

Figure 6b shows PCA of caciocavallo cheese at 60 days of maturation. The differences between two cheeses become less marked. In other words, in both cheeses the molecules produced during the ripening process become dominant compared to those coming from diet. Anyway, SILAGE cheese was characterized by flavors of moldy or smoked cheeses, whereas HAY cheese presents herbs and vinegar aromas.

4b. DISCUSSION

A main result of this trial was that no defects were found in any of the product evaluated. Certain defects (i.e. late blowing, poor taste and odour) could be recorded in cheese produced from silage diet when silage was poorly preserved (Grappin and Coulon 1996; Urbach 1990), due to the passage of butyric spores from silage to raw milk (Demarquilly, 1998).

In this trial haylage preservation quality was excellent and the nutrient supplies to animals were computed so as to be equivalent.

4.1b Chemical comp

A significant effect of Diet was observed on crude protein that was statistically higher in HAY cheese, although no differences were observed for SILAGE and HAY cheeses after brining (i.e. 0 time) (CP were 22.05±0.7 and 22.39±0.9 respectively for HAY and SILAGE cheeses). Further investigation are being carried out to study the proteolytic pattern during the aging.

4.2b Color

The color of cheese is largely influenced by animal Diet and it varies during ripening and storage according to duration, temperature and light exposure or according to contamination by pigment-producing microorganisms such as *Brevibacterium linens* (Kneifel et al., 1992). The differences in color linked to forage nature or preservation are mainly due to variable amounts of pigments (Martin et al., 2005). Carotenoids, which are the best known pigments in plants, are responsible of the yellow coloration of mainly high fat dairy products, such as butter and full-fat cheeses. Carotenoids are fat-soluble, so the yellow color is a function of both fat color and concentration (Kneifel et al., 1992). The yellow coloration is higher when animals are fed pasture or grass silage than hays, grain or maize silage diets (Martin et al., 2005), since silage making preserves carotenes better than hay making (Nozière et al., 2006). The lower yellow index (b*) of HAY cheese was probably due to a lower level of carotenoids, caused by sun drying operation during hay making. Our results are in agreement with Verdier-Metz et al. (2005) who observed a higher yellowness in SILAGE cheese. Caciocavallo cheeses lost lightness and redness throughout ripening, getting its typical yellowish color at the end of ripening. The studies concerning the influence of ripening on cheese color are not consistent, as the effect is

depending on cheese type, aging times and storage conditions used (Buffa et al., 2001; Delgado, 2011; Juan et al., 2008).

4.3bTexture

The texture of ripened cheese are mainly depend on the biochemistry of cheese ripening, and to confirm this, very limited effects of diet were observed on both instrumental and sensorial texture parameters. The proteolysis is the most complex and, in several cheese varieties also the most important, of the biochemical events that occur during ripening (McSweeney, 2004). In general terms, proteolysis leads to an increase of cheese softening due to hydrolysis of the casein matrix and through a reduction of water activity of the curd determined by changes in water binding due to the new carboxylic acid and amino groups formed on hydrolysis (McSweeney, 2004). Our results are in agreement with this observation and in accordance with the results of Delgado (2011) and Van Hekken et al. (2004).

4.4b Sensorial analysis

The chemicals that compose the total sensorial profile of cheese are coming from feeds, from preservation operations (Kalač, 2011), from inhaled air, from the digestive tract (Désage et al., 1996; Urbach, 1990), from microbiota activity in fresh milk (Martin et al., 2001). Therefore, the sensory quality of cheese depends on factors linked to both the cheese-making technology and to the chemical and microbiological characteristics of the raw milk. Nevertheless, when these factors are similar, some cheese characteristics can be associated with the feeding regimen (Martin et al., 2005). The higher yellowness of silage cheese perceived by panelists confirms the observation of others (Agabriel et al., 1999; Verdier-Metz et al., 1998; Sheath et al., 2001). Moreover, it is in accordance with the data of instrumental color and, in similarity, it can be explained by a higher carotenoid content of fat, as said before.

The flavor/odor attributes were significantly influenced by Diet, with the higher intensity of the attributes observed for SILAGE cheese as shown in Figure 2b. This fact is in accordance with the results of volatile compounds analysis. Reports of the literature about the effect of silage on sensory proprieties are not consistent. Verdier-Metz et al.(1998) observed that Saint-Nectaire type cheeses made with the maize silage was only more yellow and tended to be more bitter. Regarding goats' cheese, alfalfa hay led to cheeses with much more intense flavor than maize silage (Gaborit et al., 2002). Texture defect in cheese made using silages could be recorded when silage was poorly preserved (Grappin and Coulon, 1996; Demarquilly, 1998). Overall, according to Verdier-Metz et al.

(2005) the effect of preservation methods on sensorial proprieties of cheese could vary according to the cheese type.

The strong intensity of odor/flavor attributes at 90 d of ripening can be explained by the decrease of moisture and a relative increase of molecules that are responsible of the flavor.

The cheese become more softer at the end of ripening and this can be explained the biochemical changes in proteolysis (Martin et al., 2005).

4.5b Consumer liking

The consumers perceived differences between cheeses obtained with the two diets, at least as regards the "extremes" ripening times (30 and 90 d). In the research of Esposito et al. (2014) untrained consumers were not able to detect differences in caciocavallo ripened 90 d produced in different farms and under different management systems. Probably, the fact that many sensorial attributes varied in different way during ripening may explain this apparent inconsistency between our and the Esposito et al.'s results.

The differences observed between the scores suggest that, to improve consumer acceptability, in case of silage-based diet may be proper to extent the ripening at least 60 d, while a higher ripening time (i.e. 90 d) does not lead to an increment of acceptability of the product. Conversely, the hay cheeses ripened for 30 or 60 d are equivalent in terms of acceptability, but the most liked product is obtained by ripening the cheese for 90 d.

The slope analysis showed overall liking of caciocavallo cheese was primarily affected by taste/flavor and less by texture and appearance.

Visible characteristics, including visible fat and marbling, are thought to play an important role in orienting consumer preference before consumption for meat and meat products (Fortin et al., 2005), whereas for most other food products, such as cheese, appearance may be less important as a driver of overall liking than taste or texture (Moskowitz and Krieger, 1995).

4.6b Microbiological analysis

The results of viable counts of the microbial population during cheese making and ripening of Caciocavallo of Castelfranco are consistent with previous reports on the semi-hard pasta filata cheeses in which LAB dominated (Di Cagno et al., 2012; Di Grigoli et al., 2015; Gobbetti et al., 2002; Niro et al., 2014; Pasquale et al., 2014; Settanni et al., 2012).

In general, all the microbial groups, except for enterococci, were significantly affected by the technological process of Caciocavallo of Castelfranco, as already observed by Di Grigoli et al., (2015) in evaluating ripening time for similar pasta filata cheeses.

Moreover, during the ripening cheese pH showed a similar alteration as in the study of Albenzio et al. (2001) for Canestrato Pugliese cheese.

4.7bVolatile compounds

In agreement with others (Cifuni, 2010; Pasquale et al., 2014; Verdier et al., 1995), ketones and free fatty acids were higher in silage cheese. The less marked differences between two cheeses 60 d probably due to the fact that during the ripening of the cheese originate a large quantity of new volatile compounds that attenuate the differences caused by type of feeding.

In other words, in both cheeses the molecules produced during the ripening process become dominant compared to those coming from diet. Anyway, silage cheese was characterized by flavors of moldy or smoked cheeses, whereas hay cheese presents herbs and vinegar aromas. The moldy/smoked and herb/winegar flavors of cheeses probably, as the result of increment ester content and doubled of free fatty acids, in particularly butyric and hexanoic acids from 30 to 60 days of ripening as previous reported by Pasquale et al. (2014). This may be due to the conversion of the α-keto acids, deriving from the amino acids, to carboxylic acids via an oxidative decarboxylation (Marilley and Casey, 2004).

5b. CONCLUSION

The issue of grass preservation in the form of silage has long been a matter of debate within the traditional and the EU' labeled cheese channels, and this study examined the influence of sorghum silage on the traditional cheese Caciocavallo di Castelfranco at various ripening time. A first important results is that no defects were recorded (no blowing or poor taste and odor) in cheese produced from the two diets, and this appears to be due to the good quality of silage. The study highlighted that the use of sorghum silage sensitively modify the sensory and organoleptic characteristics of the cheeses. Many effects are due to the presence in the raw milk of compounds directly induced by feeding (carotenes and terpenes). However, several of these effects appeared different at different stages of during ripening as a results of interaction between these molecules and the formation of new compounds in cheese during ripening.

Hence, there appears the possibility to modify the perceived quality of Caciocavallo cheese by choosing the most appropriate combination of diet and ripening time. Moreover the results of the liking scores may be an useful tool to improve product acceptability according the type of forage and the ageing time.

Overall, the results obtained constitute data for the dairies to predict the evolution of products so that cheeses best reflect the originality and diversity of the area where they are produced.

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TABLES

Table 1b. Composition and chemical characteristics of HAY and SILAGE diets

	HAY	**SILAGE**
Feedstuffs (kg/head/d tq)		
Sorghum silage	-	22.0
Hay sorghum	11.0	6.0
Commercial concentrate	12.0	12.0
Chemical composition		
Dry matter kg	20.0	20.0
Crude protein kg/d	3.4	3.3
NDF kg/d	7.0	7.8
UFL n/d	17.8	17.0
Ingestion of dry matter kg/d	22.0±1.1	25.6±0.4

Table 2b. Chemical composition (%) of bulk milk from HAY and SILAGE diets.

	HAY	**SILAGE**	**SEM**	**P**
Fat	4.1	4.3	0.15	NS
Protein	3.3	3.5	0.05	0.25
Lactose	4.9	4.8	0.02	0.02

Table 3b. Chemical composition (%) of caciocavallo cheese as affected by Diet and Ripening time.

	Diet			Ripening				P		
	HAY	**SILAGE**	**SEM**	**30**	**60**	**90**	**SEM**	**Diet**	**Ripening**	**Diet*Ripening**
Dry matter	63.66	64.04	0.44	62.51[a]	62.98[a]	66.06[b]	0.54	NS	**	NS
Ash	4.28	4.62	0.08	4.31	4.43	4.61	0.10	*	NS	NS
CP (N*6.38)	26.84	25.40	0.24	25.72	26.74	25.91	0.29	**	NS	NS
Fat	20.57	20.80	1.18	18.80	21.43	21.83	1.45	NS	NS	NS

[a, b] =statistically significant difference between different ripening times

*<0.05, **<0.01, ***<0.001, NS: not significant

Table 4b. Color of caciocavallo cheese as affected by Diet and Ripening time.

	Diet			Ripening				P		
	HAY	**SILAGE**	**SEM**	**30**	**60**	**90**	**SEM**	**Diet**	**Ripening time**	**Diet*Ripening**
L*	75.5	77.4	0.92	79.37[a]	74.95[b]	75.08[b]	1.13	NS	*	NS
a*	-0.56	1.22	0.28	1.18[a]	0.93[a]	-1.12[b]	0.35	***	***	NS
b*	16.27	18.82	0.36	17.38	17.74	17.51	0.44	***	NS	NS
YI	30.82	34.73	0.44	31.23[a]	33.78[b]	33.32[b]	0.55	***	**	NS

[a, b] =statistically significant difference between different ripening times

*<0.05, **<0.01, ***<0.001, NS: not significant

Table 5b. Texture of caciocavallo cheese as affected by Diet and Ripening time

	Diet			Ripening				P		
	HAY	**SILAGE**	**SEM**	**30**	**60**	**90**	**SEM**	**Diet**	**Ripening**	**Diet*Ripening**
Hardness kg	11.47	11.20	0.83	15.46[a]	9.97[b]	8.58[b]	1.02	NS	***	NS
Springiness mm	1.67	2.13	0.16	2.24	1.80	1.66	0.20	t	NS	NS
Chewiness mm·kg	9.04	9.17	0.49	15.52[a]	7.02[b]	4.78[c]	0.60	NS	***	NS

[a, b, c] =statistically significant difference between different ripening times

P<0.05 *<0.05, **<0.01, ***<0.001, NS: not significant t: tendency

Table 6b. Descriptive attributes and definitions used to evaluate caciocavallo cheese

DESCRIPTORS	DEFINITION
APPEARANCE	
Yellow	Overall intensity of yellow color
Uniformity	Overall uniformity in structure and color
ODOR/FLAVOUR	
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
Grass	Fundamental taste of fresh grass
Hay	Fundamental taste of dry grass
TASTE	
Salty	Fundamental taste associated with sodium chloride
Sour	Fundamental taste associated with citric acid
Bitter	Fundamental taste associated with quinine
Umami	Fundamental taste elicited by certain peptides
TEXTURE	
Tenderness	Minimum force required to chew cheese sample: the lower the force the higher the tenderness
Elasticity	Degree to which the product will return to its original shape after being compressed between the teeth
Oiliness	Perception of the amount of fat released by the product during mastication

Table 7b. Sensory attributes of caciocavallo cheese from HAY and SILAGE diets at different ripening time

	SILAGE			HAY			
DESCRIPTORS	**30**	**60**	**90**	**30**	**60**	**90**	***LSD****
APPEARANCE							
Yellow	51.92^{a}	56.38^{a}*	52.08^{a}	36.04^{b}	42.38^{b}*	56.29^{a}	*7.54*
Uniformity	80.46^{c}	88.17^{ab}	68.08^{d}	92.13^{a}	84.04^{bc}	78.63^{c}	*6.15*
ODOR/FLAVOUR							
Overall Odor	53.00	63.50	59.92	60.71	58.58	53.04	/
Overall Flavor	44.00^{c}	53.75^{b}	65.25^{a}	48.75^{bc}	44.46^{c}	52.42^{b}	*6.94*
Milk	37.46	36.33	26.04	37.54	30.67	29.00	/
Butter	20.54^{bc}	26.13^{b}	34.13^{a}	18.08^{c}	17.25^{c}	22.29^{b}	*5.61*
Grass	12.83^{c}	19.54^{a}	18.21^{ab}	12.25^{c}	9.63^{c}	14.29^{ac}	*4.78*
Hay	19.13^{c}	19.67^{c}	47.29^{a}	13.79^{c}	15.33^{c}	29.63^{b}	*6.19*
TASTE							
Salty	32.13^{d}	40.25^{c}	61.42^{a}	22.83^{f}	27.50^{e}	49.33^{b}	*4.39*
Bitter	31.08^{ab}	29.00^{ab}	27.54^{b}	12.79c	24.71^{b}	34.38^{a}	*6.57*
Umami	14.790^{c}	27.83^{b}	34.67^{a}	12.33^{c}	14.46^{c}	26.08^{b}	*5.53*
TEXTURE							
Tenderness	75.00^{a}	55.13c^{d}	56.50^{c}	52.25c^{d}	48.83^{d}	64.29^{b}	*7.00*
Elasticity	39.38^{a}	39.38^{a}	22.21^{b}	45.25^{a}	39.17^{a}	30.04^{b}	*9.06*
Oiliness	36.75^{a}	24.92^{b}	41.58^{a}	22.38^{b}	22.67^{b}	36.71^{a}	*7.23*

LSD, $\alpha = 0.05$ $^{a, b, c}$ =statistically significant difference between different ripening times

Table 8b. Analysis of the effects on sensory attributes of Diet and Ripening time

Attributes	**Diet (D)**		**Ripening (R)**		**Interaction(DXR)**	
	df=1		*df=2*		*df=2*	
	F	***p***	**F**	***p***	**F**	***p***
Yellow	9.901	0.002	4.69	0.011	5.63	0.005
Uniformity	9.14	0.003	18.49	0.000	6.522	0.002
Overall Flavor	3.96	0.049	6.76	0.002	3.37	0.037
Butter	12.24	0.001	5.81	0.004	1.57	0.213
Grass	7.68	0.006	1.52	0.222	2.49	0.087
Hay	8.85	0.003	21.96	0.000	1.97	0.144
Salty	30.88	0.000	68.00	0.000	0.27	0.768
Bitter	4.60	0.034	4.52	0.013	8.771	0.000
Umami	13.81	0.000	19.65	0.000	2.08	0.129
Tenderness	3.52	0.063	3.39	0.037	5.448	0.005
Elasticity	2.83	0.095	13.79	0.000	0.82	0.441
Oiliness	7.90	0.006	12.44	0.000	2.07	0.130

Table 9b. Hedonic scores of caciocavallo cheese from HAY and SILAGE diets and ripened for 30, 60, 90 d

Samples	Average value
Hay 90d	6.63[a]
Hay 30d	6.19[b]
Silage 60d	6.19[b]
Silage 90d	6.08[b]
Hay 60d	5.87[bc]
Silage S30	5.73[c]
LSD (5%)	0.34

[a, b, c] =statistically significant difference between different ripening times

Table 10b. Result of slope analysis

.

	Average	
Sensory preferences	**Real slope**	**Normalized slope**
Aspect	0.66[b]	0.24[b]
Flavor	0.83[a]	0.51[a]
Texture	0.57[b]	0.25[b]
F-Value	4.86	6.18
***P*-value**	0.004	0.003
LSD*	0.15	0.18

[a, b] =statistically significant difference between different ripening times

Table 11b. Evolution of microbial populations (log CFU/ml or g) and pH during manufacture and ripening of Caciocavallo of Castelfranco

Growth media[2] and pH	Diet														Statistical significance[3]		
	H Samples[1]							S Samples[1]									
	M	NWC	CC	CS	C1	C30	C60	M	NWC	CC	CS	C1	C30	C60	F	L	F*L
PCA	5.62±0.28	nd	nd	nd	nd	nd	nd	5.81±0.45	nd	nd	nd	nd	nd	nd	ns	-	-
mMRS 25°C	4.86±0.38^{a}	4.61±0.78^{a}	4.89±0.55^{ab}	4.86±0.45^{a}	4.82±0.44^{a}	7.03±0,84^{cd}	7.92±0.64^{d}	5.57±0.31^{abc}	4.61±0.81^{a}	5.55±0.25^{abc}	4.78±0.75^{a}	4.33±0.58^{a}	5.43±0.65^{abc}	6.57±0.46^{bcd}	*	***	*
mMRS 37°C	5.33±0.21^{abc}	5.57±0.45^{abc}	5.38±0.44^{abc}	5.49±0.27^{abc}	5.53±0.32^{abc}	6.78±0.99^{cd}	7.58±0.41^{d}	5.64±0.34^{abc}	5.04±0.61^{ab}	5.59±0.23^{abc}	5.71±0.46^{abc}	4.44±0.63^{a}	5.59±0.63^{abc}	6.48±0.70^{bcd}	**	***	ns
M17 30°C	6.03±0.33^{b}	8.50±0.18^{a}	6.53±0.20^{b}	7.75±0.33^{abc}	8.03±0.60^{ac}	7.55±0.60^{abc}	6.99±0.85^{bc}	6.08±0.32^{b}	8.44±0.15^{a}	6.63±0.25^{b}	8.44±0.60^{a}	8.55±0.79^{a}	7.51±0.68^{abc}	6.84±0.99^{bc}	ns	***	ns
M17 42°C	5.97±0.40^{c}	8.48±0.06^{a}	6.56±0.09^{ce}	8.16±0.27^{abd}	8.01±0.23^{abd}	7.50±0.65^{bdf}	5.80±0.50^{c}	6.01±0.45^{c}	8.29±0.36^{ab}	6.59±0.16^{cef}	8.83±0.66^{a}	8.38±0.16^{ab}	7.37±0.82^{def}	6.46±0.93^{c}	*	***	ns
S-B	4.92±0.58	4.04±0,43	4.79±0.39	3.82±0.20	5.08±0.05	4.40±0.47	4.20±0.32	5.06±0.43	5.60±0.59	5.57±0.24	3.40±0.40	3.98±0.36	3.74±0.88	3.99±0.91	ns	ns	ns
DRBC	nd	nd	nd	nd	3.22±0.59^{ac}	3.51±0.23^{ab}	3.83±0.58^{ab}	nd	nd	nd	nd	2.18±0.61^{c}	3.91±0.40^{ab}	4.29±0.47^{b}	ns	***	ns
TBX	1.38±0.99^{abc}	1.17±0.23^{ab}	1.21±0.22^{ab}	2.06±0.58^{bcd}	<1^{a}	<1^{a}	<1^{a}	1.72±0.61^{abc}	2.11±0.40^{cd}	1.68±0.26^{abc}	2.68±0.47^{d}	<1^{a}	<1^{a}	<1^{a}	**	***	ns
pH	6.79	4.44	6.70	5.11	5.28	5.22	5.21	6.74	4.49	6.76	5.33	5.29	5.29	5.39	-	-	-

The values are the means ± SD obtained from duplicate plates of three independent trials. Means within a row with different letters (a to f) are significantly different ($P < 0.05$); nd, not determined

1: H, hay; S, silage; M, milk; NWC, natural whey culture; CC, curd immediately after coagulation; CS, curd at end of acidification; C1, C30 and C60, cheese after 1, 30 and 60 days of ripening

2: PCA for mesophilic bacteria; mMRS 25°C for mesophilic bacilli LAB; mMRS 37°C for thermophilic bacilli LAB; M17 30°C for mesophilic cocci LAB; M17 42°C for thermophilic cocci LAB; S-B for enterococci; DRBC for yeasts and moulds; TBX for *Escherichia coli*.

3: F, feeding; L: sample; F*L: interaction; P value: ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$; ns, not significant; -, not determined

FIGURES

Figure 1b. Flow diagram of traditional Castelfranco caciocavallo cheesemaking

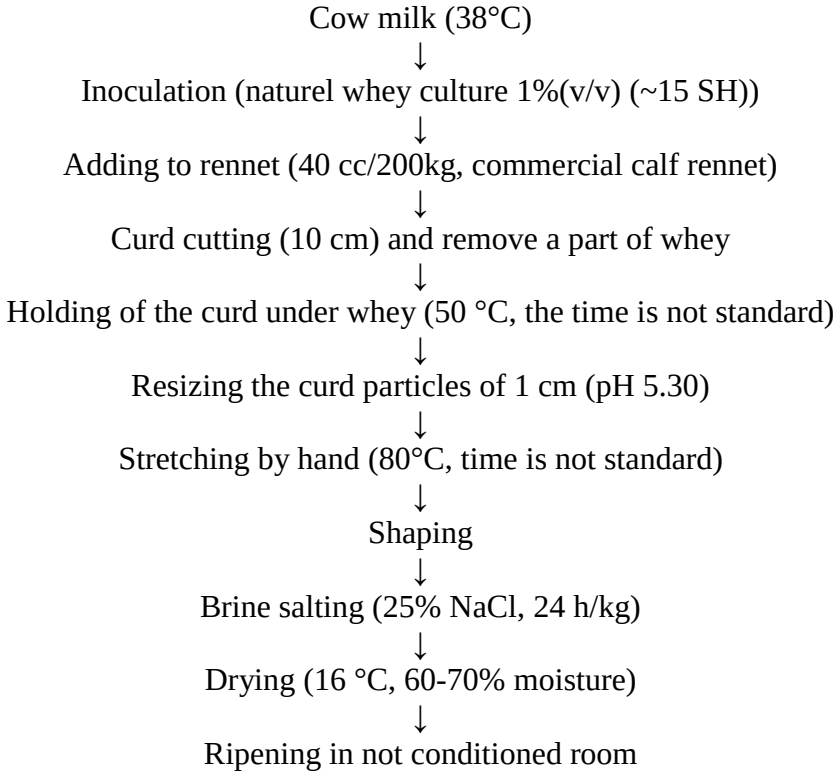


Figure 2b– Sensory profile graphic of the caciocavallo cheese for HAY and SILAGE based diets

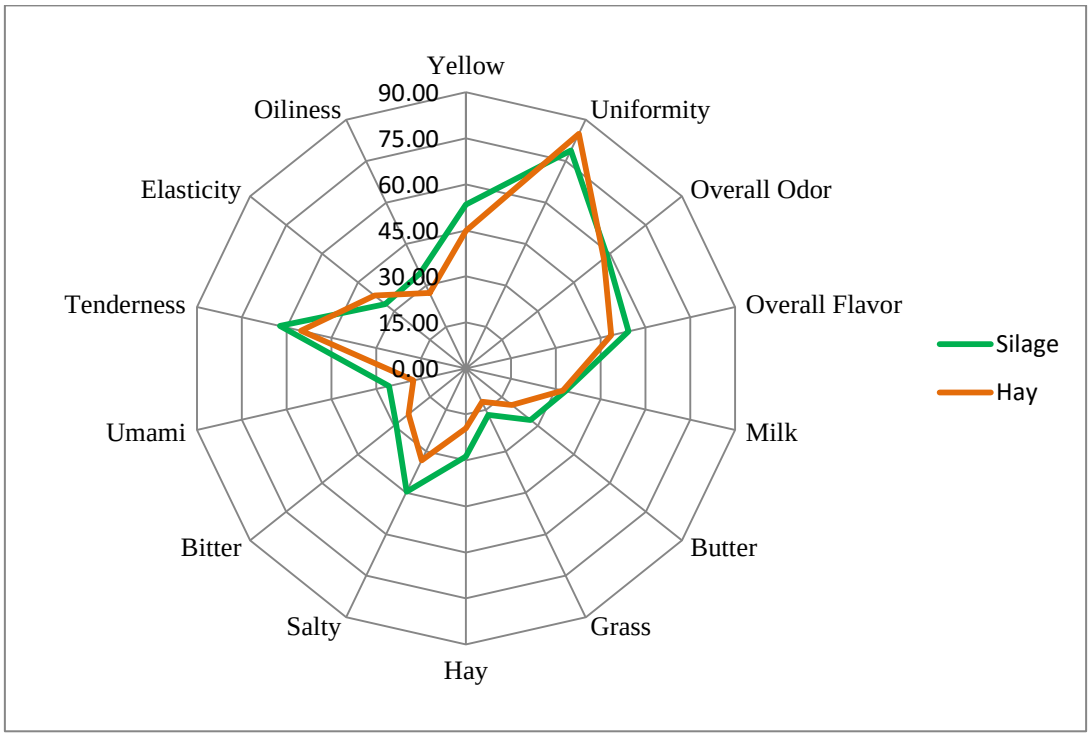
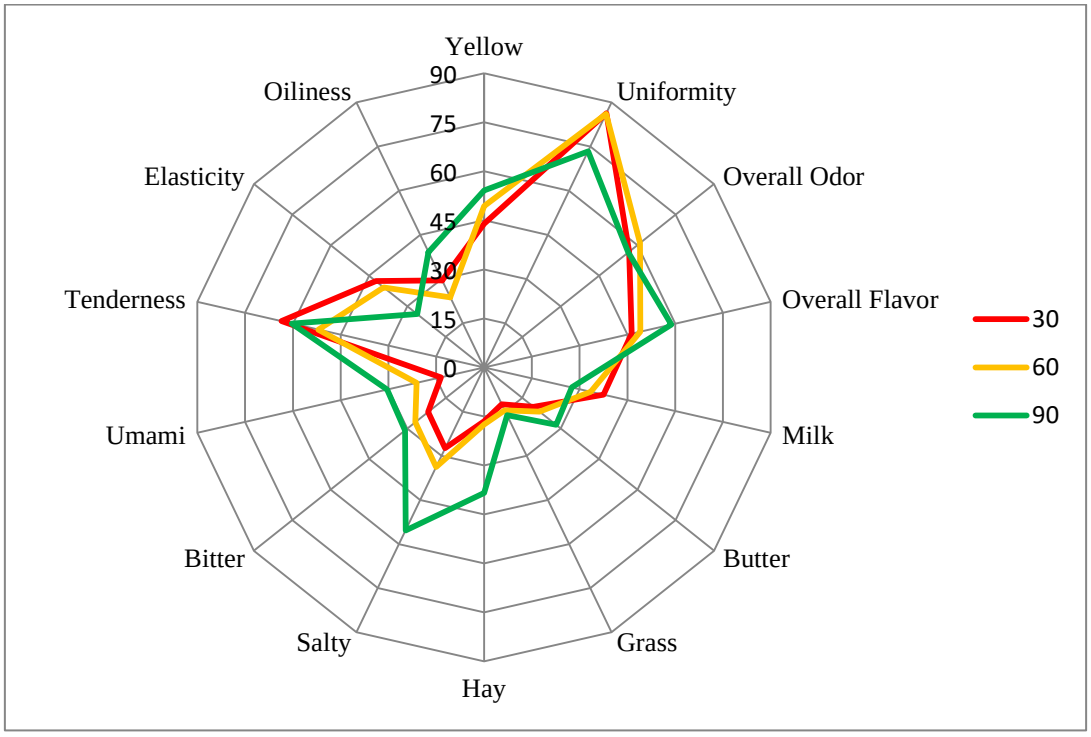


Figura 3b – Sensory profile graphic of the caciocavallo cheese at different time of ripening



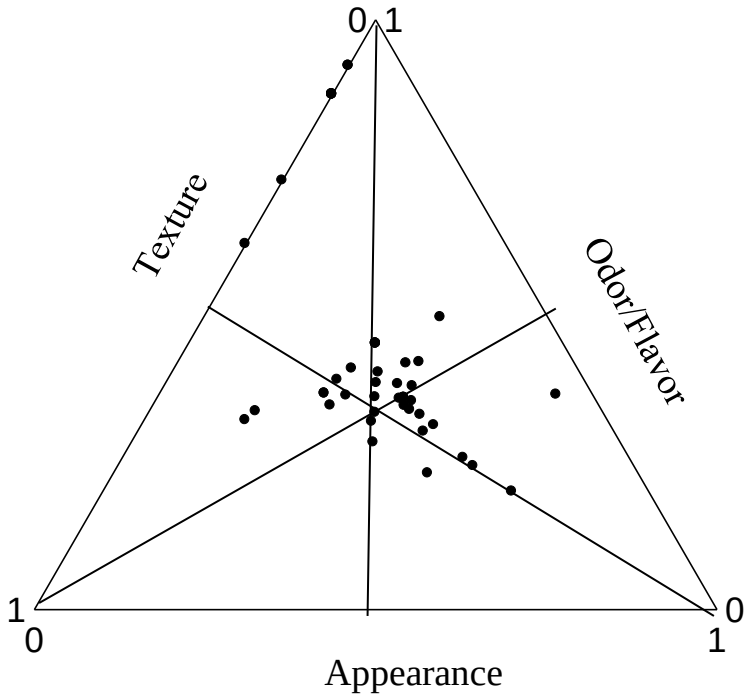


Figure 4b. Triangle plot of normalized slopes

Figure 5b**.** PCA of caciocavallo cheese at 30 days of Ripening produced with milk from cows fed HAY or SILAGE based diets

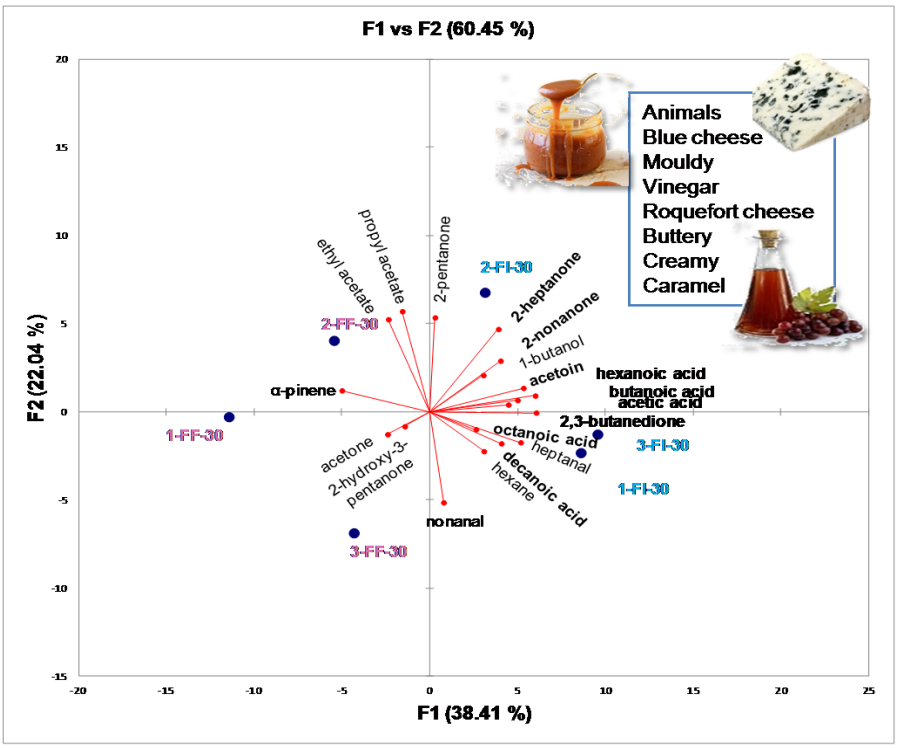
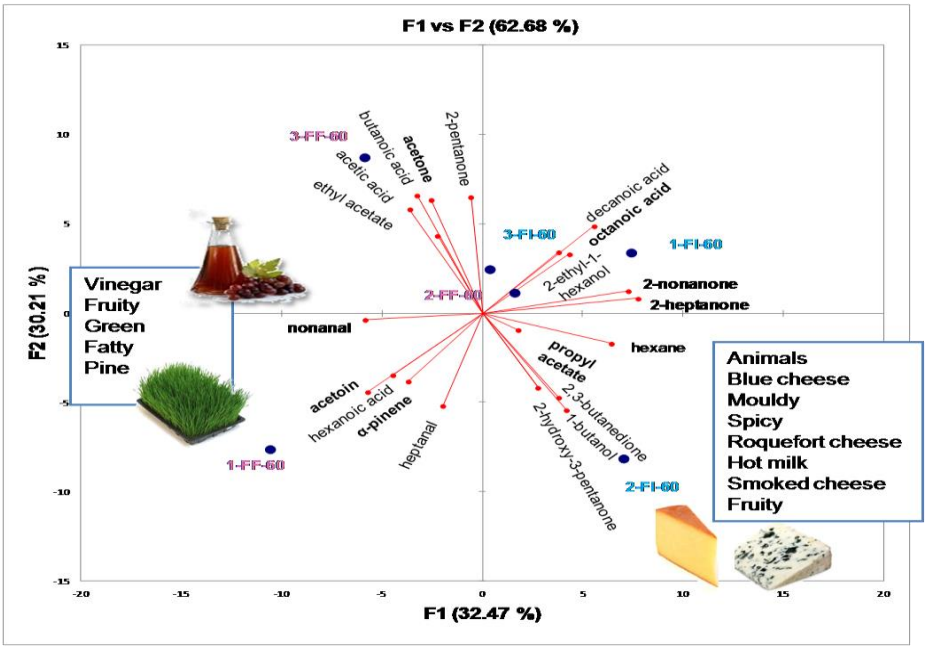


Figure 6b. PCA of caciocavallo cheese at 60 days of Ripening produced with milk from cows fed HAY or SILAGE based diets



EXPERIMENT C

INFLUENCE OF INCLUSION OF FRESH FORAGE IN BUFFALO COWS' DIET ON QUALITY OF MILK AND MOZZARELLA CHEESE

1c. INTRODUCTION

In recent years, a great deal of interest has been focused on fat content and fatty acid (FA) profile of foods, due to growing awareness about their roles on human health. The guidelines of World Health Organization (WHO, 2003) indicate that the unsaturated fatty acids (UFA) may reduce the risk of many diseases such as heart and cardiovascular diseases, cancer, obesity, diabetes (Ulbricht and Southgate, 1991). As a group of UFA, polyunsaturated fatty acids (PUFA) are of particular interest because of some specific potential benefits to human health (Blankson et al., 2000; Lock and Bauman, 2004). Some PUFA, vaccenic acid, rumenic acids, linolenic and conjugated linoleic acids (CLA, a series of positional or geometrical isomers of linoleic acid, cis-9, cis-12 octadecadienoic acid,), have shown additional positive health effects (Elgersma et al., 2004). Particularly, CLA has identified as the only known fatty acid able to inhibit cancer in experimental animals and to prevent mammary glands and skin tumors in experimental animals (Elgersma et al., 2004). Some isomers of CLA have also been linked to enhancing growth of lean body mass (Lock and Bauman, 2004; Park et al., 1983).

Milk fat typically contains, largely as a consequence of microbial biohydrogenation in the rumen, a high proportion of saturated (SFA) and monounsaturated fatty acids (MUFA) and small amounts of PUFA (Dewhurst et al., 2006; Lock and Shingfield, 2004). Myristic (C14:0) and palmitic acids (C16:0) are two major SFA in milk and have been implicated in increasing total and low density lipoprotein (LDL) cholesterol concentrations (Lock and Shingfield, 2004; Williams, 2000), causing a negative consumer perception and a public health concern (White et al., 2001).

Fatty acid composition of dairy products can be significantly improved through nutrition of the dairy cows (Lock and Bauman, 2004; Lock and Shingfield, 2004). Forages provide a low-cost approach in comparison with diet supplementation strategies, such as oils and starch, which are designed to improve milk fatty acid profiles relative to their impacts on the health of humans (Dewhurst et al., 2006). The usual total FA content in forages ranges between 20–50 g/kg dry matter (DM). In temperate countries, as an instance, fresh grass contains 1–3% FA and about 55–65% of these FA are composed of α-linolenic acid (Bauchart et al., 1984)

Plants have unique ability to synthesize the α- linoleic acid which is the building block of the omega - 3 series of essential fatty acids and elongation and desaturation of this fatty acid results in the synthesis of EPA and DHA (Scollan et al., 2006).

It is important to increase and protect the level of the UFA in the forages to transfer them to dairy products. Fatty acid content of forages can be affected by many factors such as plant species, cultivar, climate, day length, rainfall, fertilization and stage of growth (Kalač and Samková, 2010). The highest fatty acid content is in young plants at the first cut, then it decreases during the growth cicle, particularly around flowering. To feed a larger numerous of animals and to gain feedstuff all year round, fresh forages are treated in different ways as wilting, ensiling, drying treatments. As a result of these treatments, mechanical damages of plant tissues combined with air access determine major changes of the fatty acid profile of forages and an extensive oxidation of unsaturated fatty acids. Significantly higher oxidative loses of α- linoleic acid were observed in hay as compared with silage (Aii et al., 1988).

Forages may have a great effect also on sensorial properties of the dairy products. Although, little is known about the relative importance of grassland type and the associated mechanisms for cheese sensory properties, feeding is one of the most important factor that may affect sensorial properties of dairy products either positively or negatively (Farruggia et al., 2014). The major differences were observed when the animals are fed on pasture or with fresh forages (Metz Verdier et al., 2000). Utilizing fresh forages in the animal rations has an effect the dairy products color due to the higher content of the natural pigments. Feeding forages increases the content of β-caroten and carotenoids, vitamin A and tocopherols in milk and other products (Nozière et al., 2006).

Metz et al. (2000) observed that the cheese made with milk from pasture generally is yellower, with a less firm texture, has stronger taste and is less piquant, less sour and less fruity than those made with winter milk. Furthermore, some studies showed that cheese originating form mountain pastures were rich in volatile compounds such as terpenes and sesquiterpenes (Dumont, 1981; Urbach, 1990). Determining the relationship between green forages and sensory properties of dairy products getting to be more important especially for the products have been granted PDO status.

2.c AIMS

Buffalo farming is a dairy enterprise emerging in Italy and other European and non-European countries due to progressive saturation of the dairy marketplace that requires product diversification. In such increasingly competitive market of dairy products, buffalo dairy farms are forced to pursue competitive strategies focusing on product quality.

Dietary recommendations for human health indicate a reduction of fat intakes, in terms of saturated and trans fatty acids, and of fat of animal origin. Fat of milk and dairy products has a low content of polyunsaturated fatty acids (PUFA), but it contains the health-promoting rumenic acid (cis-9, trans-11 C 18:2, commonly referred as CLA), a naturally occurring anticarcinogen. Therefore, there is a growing interest in the development of dairy products naturally enriched in PUFA and CLA.

Manipulation of milk fatty acid composition through nutrition strategies has been an important target for the dairy farming. Forages provide a low-cost approach in comparison with diet supplementation strategies, such as oils and starch, and they do not result in large increases in trans 18:1 isomers other than trans-11 18:1.

The present study aimed to evaluate the effect of feeding fresh sorghum on composition, fatty acid profile, color, sensory properties, and consumer liking of "*Mozzarella di Bufala Campana*" PDO cheese.

3c.MATERIAL AND METHOD

3.1c Experimental Design

The experiment took place in a buffalo dairy farm (UAA about 154 ha) located in the Sele plain, Campania region, southern Italy. The farm raised 368 dairy buffalo cows and produced *Mozzarella di Bufala Campana* cheese exclusively from milk produced on farm. Other relevant characteristics of the farm are given in Table 1a of Chapter 1.

Two homogenous groups of 16 lactating buffaloes (on average, milk yield 13.3±1.5 kg/d, days in milk 110±13 d, parity 3.0±1.3) were fed two total mixed rations containing (FRESH group) or not (DRY group) 20 kg of fresh sorghum (Table 1c). The diets were isonitrogenous, and isocaloric (Table 2c). The groups were housed in two adjacent free stall pens with concrete floor and equipped with feed manger, drinker, and external paddock. All animals were handled in similar way in terms of feeding and management and were milked twice daily (05:00 and 17:00). Cows were fed once daily (08:00 h) in amount to provide approximately 10% orts for ad libitum consumption. The ration was re-approached several times daily to ensure unlimited access to feed.

After 10 days of adaptation to diets, on three days, milk from each cow was sampled along with the collection of bulk milk from DRY and FRESH groups. The two batch of milk (on average 200 kg) were used to produce mozzarella PDO cheese, at the same time, in separate vats. In each manufacturing day, two batches of about 20 kg of mozzarella, in pieces of 50 g, were produced. Figure 1c presents mozzarella cheese making process

3.2c Sampling procedure

Milk pH and whey pH (after coagulation and cutting) were measured every 30 min during the cheese making process.

For each day of cheese making, individual and pooled bulk milk were sampled, packed in multiple 200-mL plastic flasks and, under refrigeration, sent to the laboratory. Moreover, feed intake of the two groups was measured by the difference between feed administered and orts. Samples of each rations were collected separately for each day, dried in oven (65ºC) with forced ventilation to measure dry matter content. Subsequently, samples were unified per diet, grinded in a mill equipped with a wire mesh of 1 mm, and stored until analysis.

Immediately after production, the mozzarella was packaged in plastic bags (1 kg each) containing diluted brine solution (preserving liquid) and sent to laboratory. The day after cheese making, the physicochemical, color, texture and sensorial analyses were performed.

3.3c Chemical analysis, color and texture

Chemical analysis of feed, milk and cheese were determined as previously described for experiment b. Color was determined on samples taken from external and internal parts of 4 cylindrical pieces (diameter, 23 mm; height, 11 mm) of mozzarella. In each test, 4 replicates were performed and the mean of four replicates was used for statistical analysis. The analysis were carried out during 3 d from manufacturing (i.e. until corresponding to the end of shelf-life) and the differences of overall color (El-Nimr et al., 2010) between the days of analysis (i.e. Delta 1-2 and Delta 1-3) were calculated. The condition of analysis and instruments details were similar as previously described for experiment b.

To determine fatty acid profile, lipid extraction from mozzarella was carried out by the Folch et al. (1957) method. Duplicate samples of extract were methylated by adding 300 ml of 2N methanolic KOH to a 1 ml of %5 fat/hexane mixture. Gas-chromatographic analysis of fatty acids 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 lm). Operating conditions were: a helium flow rate of 1.2 ml/min.; a FID detector at 240 °C; a split–splitless injector at 280 °C with an injection rate of 144 ml/min and an injection volume of 1 ll. The temperature program was 5 min at 80 °C, and increase to 165 °C at 5 °C/min, 5 min at 165 °C and a subsequent increase to 260 °C at 3°C/min. Retention time and area of each peak were computed using the Clarity software. Fatty acid peaks in chromatograms were identified using the Supelco 37 Component FAME MIX (SupelcoBellofonte, PA, USA) and a CLA isomer mixture (Nu-Chek-Prep., Inc. Elysian, MN, USA) as external standards. A butter oil reference standard (CRM 164; Commission of the European Community Bureau of Reference, Brussels, Belgium) was also analyzed periodically to control for column performance, to obtain response factors and to convert-areas of individual FAs into a weight percentage of the total fat. Fatty acids were expressed as percent of total methylated fatty acids.

3.4c Sensorial analysis

The sensory analysis of mozzarella cheeses was carried out by a panel composed of 10 trained assessors. The consumer acceptability was assessed by a panel composed by 94 consumers (49 females and 45 males) with an age ranging from 24 to 60 years. The condition of analysis were similar as previously described for experiment b.

3.5c Statistic

All statistical analysis were done by using SAS package software. The effect of the two diets (FRESH and DRY) on chemical composition, texture, color and fatty acid profile was evaluated by using one-way ANOVA.

Sensory profile data were subjected to ANOVA with assessor (10), replication (3), product (2), and their interactions as factors. A further ANOVA was performed using diet (2), as factors. To identify the most liked product, acceptability data were analyzed by ANOVA, using diet as factor. The relationship between overall liking and attribute liking (appearance, taste/flavor, and texture) was analyzed by linear regression analysis according to the procedure described in experiment b.

4c. RESULTS

4.1c Milk traits and chemical composition and color of mozzarella

Tables 3c and 4c show, separately for DRY and FRESH groups, milk traits, cheese yield (table 3c) and chemical composition of mozzarella (table 4c). No effects of diet were observed for any parameters ($P>0.05$).

Table 5c presents the color of mozzarella. No significant differences were observed between FRESH and DRY mozzarella ($P>0.05$). The b*, Chroma and Yellow Index values differed ($P<0.001$) between the internal and the external area of mozzarella as effect of different texture (data not shown).

4.2c Fatty acids

Fatty acid profile of mozzarella cheese of the two dietary groups is presented in Table 6c. DRY mozzarella cheese had significantly higher values of C4:0 ($P<0.05$), C14:0, C14:1 and C16:0 FA ($P<0.01$). In regard to this component the difference was, on average, 4.6 percentage points. On the other hand, significantly higher values in FRESH mozzarella were observed for C18:1n9c ($P<0.001$) C18:2n6c ($P<0.01$), TVA ($P<0.05$), CLA and C18:3n3 ($P<0.001$). As consequences, FRESH mozzarella had significantly ($P<0.001$) higher levels of PUFA and UFA, lower percentages of SFA and a minor values of AI.

4.3c Sensorial properties

The first phase of quantitative descriptive analysis, the generation of the terms, allowed the development of a common vocabulary of descriptive terms for mozzarella di buffalo cheese. Sensory descriptors and their definition are shown Table 7c. The trained panel rated the intensity of 19 attributes to describe the aspect (three attributes), odor and flavor (six), taste (three), and texture (seven) of the products.

A main result of the sensory analysis was that the product × replication and product × assessor interactions were never significant ($P > 0.05$) or very little compared to the effect samples, indicating a high reliability of the panel performance. That is, the cheeses were not evaluated differently in different replications or by different assessors. The lack of significant interactions allowed us to perform a second ANOVA by using diet as factor. According to this analysis, most of the sensory attributes were influenced by diet, except cream color (aspect), butter and yogurt (odor/flavor) and salty (taste) (Table 8c Figure 2).

In particular, the product FRESH, differed from product DRY for:

- Appearance: it was brighter and smoother.

- Odour / flavor: presented lowest intensity of the overall odor, overall flavor, milk and whey O/F.

Taste: it was sweeter and more sourer.

Texture: it was more peelable, more tender and juicier, but it was less elastic and cohesive. Moreover, it presented less resistance to chewing and during chewing was characterized by a lower intensity of stridor.

4.4c Consumer liking

The overall preference were similar for FRESH and DRY products (Table 9c). For both FRESH and DRY mozzarella the values were higher than 5, neutral value. The slope analysis did not shown any significant differences between the specific sensory inputs (appearance, taste/flavor and texture) to drive the overall liking (Figure 3)

4.5c Volatile compounds

Figure 3c shows PCA of FRESH and DRY buffalo milk. The bold characters indicate significant differences between groups. The FRESH milk was characterized by a higher content of aldehydes in particular, hexanal, the molecule responsible of herbaceous and green apple flavors. Moreover FRESH milk had higher content of toluene and 2-ethyl –1 hexanol. The DRY milk was characterized by a flavor of smoked/moldy cheese.

Figure 4c shows PCA of FRESH and DRY mozzarella. The FRESH samples had higher herbaceous flavor with alcoholic nuances caused by the presence of pentanol. Conversely, fruity and butter flavors predominated in DRY mozzarella due to the higher presence of ethyl esters (propyl propanoate and propyl butanoate). It is noticeable that, although higher than in milk, the ester content in mozzarella is rather low.

5c. DISCUSSION

The main objective of this study was to verify if the use fresh forage daily cut from field could be a simple way to modify FA composition of mozzarella cheese under conditions of intensive farming. Our results show that the objective was reached, since the FA acid profile of FRESH mozzarella was significantly improved by feeding lactating buffalo cows with fresh sorghum. This result is in agreement other studies in which an improvement of the fatty acid profile of the milk was observed by using grazing pasture (Collomb et al., 2002; Falchero et al., 2010; Esposito et al., 2014). Feeding forages to ruminants increases the n-3 polyunsaturated fatty acid (PUFA) content in milk (Dewhurst et al., 2003c, 2006) as they are natural rich sources of C18:3 n-3. The importance of using fresh forages in animal diet is due to higher loose and oxidation of unsaturated fatty acids during the forage processing operations (Scollan et al., 2006). In this respect, it is not surprising that cheese from animals fed with high dietary forage proportions, have higher proportions of PUFA and particularly omega 3 fatty acids (FA), versus 'conventional' cheese (Ellis et al., 2006; Fievez and Vlaeminck, 2006; Lourenço et al., 2008). Chilliard et al. (2007) summarized the effects of offering fresh grass or mixed winter diets to dairy cows and noted that fresh pasture increased the concentration in cow milk fat of C18:0 (+2 g/100 g total FA), 18:1c-9 (+8), C18:2 (+1) and CLA (+0.6), while decreasing the concentration of 16:0 (−13 g/100 g total FA) (Woods and Fearon, 2009). The type and source of dietary carbohydrate may influence rates of microbial fermentation in a way that alters the rate of CLA production or utilization by rumen microbes and ultimately the concentration of CLA in milk fat. Such an effect could help explain the reported higher differences in the CLA content of cheese fat observed between cows fed with and without fresh forage (Dhiman et al., 1999). Sugars, such as fructosans, starch, pectins, and soluble fiber content, greatly decline during the fermentation process used to preserve forage (Soest, 1994). Furthermore, during the drying process necessary to obtain hay, the forage is subject to respiration, which can lead to a 10 to 20% decrease in certain constituents, mainly, the carbohydrate fraction of the plant cell contents. Thus, the high concentrations of rapidly fermentable starch, sugars, and soluble fiber that are found in immature spring pastures may create a rumen environment and conditions that favor a greater production or a reduced utilization of CLA by the rumen bacteria (Kelly et al., 1998).

The effects of the dietary treatment on sensory profile were more evident for flavor/odor and texture attributes of mozzarella. The sensory quality of cheese depends on several factors linked to both the cheese-making technology (milk treatment, starter cultures, clotting process

conditions) and the chemical and microbiological characteristics of the raw milk (Martin et al., 2005). Nevertheless, when other factors are similar, some cheese characteristics can be associated with the feeding regimen. Studies on the characteristics of cheeses from animals kept at pasture showed relative differences of color, flavor and texture (Bonanno et al., 2013; Chilliard and Ferlay, 2004; Esposito et al., 2014). Changes in the texture of FRESH mozzarella may be attributed, at least partly, to the corresponding FA composition, which was poorer in SFA such as C16:0 and richer in unsaturated FA such as C18:1. The lower melting point of unsaturated fatty acids tend to produce more soft cheeses (Martin et al., 2005). The higher flavor/odor attributes of DRY product can be explained to the fact that fresh sorghum partially replace meadow haylage that is characterized by a high content of volatile compounds. Higher levels of flavor/odor attributes were also observed in caciocavallo produced by using a silage based diet. It should be noted that several sensory characteristics related to animal feeding are more evident in more aged cheeses as results of interaction between compounds from feeding and the new molecules originated from lipolysis and proteolysis (McSweeney, 2004).

In regard the volatile compounds, the higher content of hexanal which is responsible of herbaceous and green apple flavor, in FRESH milk may be due to the higher content of PUFA in green forages (Villeneuve et al., 2013), from which, via lipoxygenase, originate the aldehydes. The smoked/moldy cheese flavor of DRY milk, probably due to a higher free fatty acids content. According to Chilliard and Lamberet (1984) dietary fresh forages reduces the lipolysis activity at in gastric level and, consequently, the free fatty acids content. Moreover DRY milk showed more abundant ketones, in agreement with previous studies (Villeneuve et al., 2013).

The FRESH mozzarella cheese samples had higher herbaceous flavor with alcoholic nuances caused by the presence of pentanol. This alcohol originates via alcohol dehydrogenase from pentanal (aldehyde) which, in turn, can derives from linoleic acid of fresh forage (Pasquale et al., 2014; Villeneuve et al., 2013).

Conversely, fruity and butter flavors predominated in DRY mozzarella due to the higher presence of ethyl esters (propyl propanoate and propyl butanoate). The ethyl esters could originate via esterification of free fatty acids with alcohols either in mammal gland (Moio et al., 1993) or from enzymatic activity of lactobacilli during cheese making process (Hosono et al., 1974). This last hypothesis appears to be the most likely since DRY milk showed a higher content of free fatty acid but not of esters. The higher ester content of milk more than

mozzarella cheese, probably can explain, the high temperatures (90°C) reached during the cheese-making process may have reduced number and activity of Lactobacilli.

In conclusion with respect to mozzarella cheese, milk samples have grater variability of differences in volatile compounds. Probably, this is due to bacterial origin volatile compounds in mozzarella cheese that attenuate the differences comes from different diet.

Milk contains variable amounts of pigments such as carotene, which is present in large proportions in green forage and contributes to the yellow coloration of dairy products (Nozière et al., 2006). These compounds are extremely sensitive to ultraviolet (Park et al., 1983) and are degraded during drying, decreasing with increasing of light exposure level. Therefore, the type of diet has a marked effect on the color of the cheese which presents a much more yellow color if diet is based on fresh forages (Martin et al., 2005).

The lack of significant differences in the color of FRESH and DRY mozzarella is due to the fact that in milk and buffalo cheese (as well as in milk and cheese from sheep and goat), beta carotene is below an analytically-determinable level since it is completely metabolized in the liver (Bergamo et al., 2003). Dietary beta carotene is deposited in fatty tissues and liver and, in part, may get to milk fat globules. The animal is able to convert, according to the physiological needs, a certain amount of beta carotene to retinol (vitamin A). Compared to cows, goats have a more efficient enzymatic conversion system of the beta carotene into retinol and, thus, the presence of beta carotene in goat adipose tissue is very small. Probably the same physiological mechanism is on the basis of the absence of analytically detectable beta carotene in milk of sheep and buffalo (Mora et al., 2000). The results of instrumental color are confirmed also by sensorial analysis, since no differences for cream color was observed by the panelists.

Although the FRESH and DRY mozzarella were well differentiated, the consumer panel did not perceive any differences for mozzarella produced by using fresh sorghum. However, this fact per se is not negative on the contrary, it can be a strong point in the traditional market of *Mozzarella di Bufala Campana* (De Stefano, 2004).

6c. CONCLUSION

The inclusion of at least 20 kg of fresh forage in the diet of lactating buffaloes allows an improvement of the fatty acid profile of the mozzarella with an increase of the content of PUFA and CLA. While modifying the sensory profile and the volatile fraction of the mozzarella, the use of fresh fodder does not change the product acceptability of *Mozzarella di Bufala Campana*. Efforts should be made by producer to signal the quality of this product to the consumer to improve its recognition.

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TABLES

Table 1c. Composition (kg/d tq) for two experimental diet.

	DRY	FRESH
Maize silage	18	15
Fresh sorghum	-	20
Meadow haylage	10	4
Alfalfa hay	3	1.5
Meadow hay	1.5	1
Maize mash	2.5	2.5
Maize meal	1.5	1.5
Soybean meal	1.8	2.1
Sunflower meal	0.5	0.6
Barley meal	0.3	0.3
Bicarbonate	0.2	0.2
Vitamin and mineral supplement	0.05	0.05

Table 2c. Chemical and nutritional characteristics for two experimental diet.

	DRY	FRESH
DM kg	17.9	17.97
UFL/kg DM	0.84	0.86
CP % DM	14.2	14.9
NDF % DM	42.2	45.1
ADF % DM	24.18	25.97
Ash % DM	6.80	7.06
Starch % DM	22.28	20.75

Table 3c. Chemical characteristics and production of milk for DRY and FRESH groups.

	DRY	FRESH	SEM	P
Milk production kg/d	8.0	8.4	0.29	NS
Fat %	9.5	9.1	0.33	NS
Protein %	5.1	5.3	0.10	NS
Lactose %	4.7	4.7	0.071	NS
Mozzarella Yield %	24.7	24.6	0.5	NS

Table 4c. Chemical characteristics of DRY and FRESH mozzarella cheeses.

	DRY	FRESH	SEM	P
Dry matter %	47.5	47.8	4.02	NS
Fat % DM	57.6	56.8	0.98	NS
Protein % DM	25.3	24.9	0.78	NS

Table 5c. Color of DRY and FRESH mozzarella cheeses

	DRY	FRESH	SEM	P		
				Diet	Area	Diet*Area
L*	93.3	93.7	1.44	NS		NS
a*	-2.69	-2.50	0.17	NS		NS
b*	8.71	7.71	0.42	NS	***	NS
Hue	179.05	170.59	6.81	NS		NS
Chroma	9.14	8.13	0.40	NS	***	NS
Delta E 1-2	2.30	2.70	0.56	NS		NS
Delta E 1-3	3.50	3.11	0.54	NS		NS
Yellow index	13.36	11.76	0.60	NS	***	NS

Delta E 1-2 differences in overall color between the 1st and the 2nd d of analysis

Delta E 1-3 differences in overall color between the 1st and the 2nd d of analysis

*** P<0.001 NS: Not significant

Table 6c. Fatty acid composition (g/100 g of fat) of DRY and FRESH mozzarella cheeses

Item	DRY	FRESH	SEM	P
C4:0	3.26	2.9	0.114	*
C6:0	2.29	2.4	0.081	NS
C8:0	1.13	1.1	0.072	NS
C10:0	2.26	2.5	0.106	NS
C11:0	0.11	0.16	0.027	NS
C12:0	3	2.9	0.114	NS
C13:0	0.11	0.1	0.045	NS
C14:0	11.8	9.6	0.239	**
C14:1	1.12	0.37	0.053	**
C15:0	1.19	1.34	0.047	NS
C16:0	38.9	34.3	0.384	**
C16:1	2.9	3.04	0.093	NS
C17:1	0.46	0.51	0.041	NS
C18:0	9.46	11.1	0.243	NS
C18:1n9trans	0.8	1.12	0.021	*
C18:1n9cis	18.3	22.3	0.505	***
C18:2n6cis	1.59	2.3	0.127	**
C20:0	0.22	0.25	0.009	NS
CLA	0.34	0.92	0.044	***
C20:1	0.44	0.55	0.038	*
C18:3n3	0.2	0.6	0.037	***
C22:0	0.12	0.14	0.006	NS
C20:3n3	0.08	0.09	0.536	NS
C20:4n6	0.03	0.07	0.507	NS
C22:2	0.03	0.04	0.126	NS
C24:0	0.05	0.09	0.084	NS
C24:1	0.03	0.04	0.114	NS
C22:6n3	0.03	0.04	0.081	NS
SFA	73.6	69.7	0.536	***
Unsaturated fatty acids	26.5	30.3	0.507	***
PUFA	2.2	3.66	0.126	***
Atherogenic index[1]	3.35	2.62	0.084	***

*P<0.05;**P<0.01;***P<0.001 NS: Not significant

[1][C12:0 + (4 × C14:0) + C16:0]/unsaturated FA

Table 7c. Descriptive attributes and definitions used to evaluate Mozzarella cheese

DESCRIPTORS	DEFINITION
APPEARANCE	
Cream color	Intensity of the mozzarella cream color
Brightness	Amount of light reflected from the product's surface
Smoothness	Product surface free from holes and granules
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	Aromatics associated with whey
Yogurt	Characteristic 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	
Peelability	Separation of skin during the mastication
Tenderness	Minimum force required to chew cheese sample: the lower the force the higher the tenderness
Elasticity	Degree by which original shape of a product is restored after compression between the teeth
Juiciness	Wet sensation in the mouth caused by a product after compression between the teeth
cohesiveness	The degree to which a cheese sample holds together or adheres itself after chewing
Chewiness	The total amount of energy required to masticate the sample to a state pending swallowing.
Harshness	Ability to produce noise when chewing

Table 8c. Sensorial profile of DRY and FRESH mozzarella cheeses.

	FRESH	DRY	*LDS*
ASPECTS			
Cream color	74.95	74.1	6.15
Brightness	72.15[a]	65.30[b]	6.70
Smoothness	74.00[a]	61.55[b]	6.11
ODOR and FLAVOR			
Overall Odor	74.80[b]	81.45[a]	5.28
Overall Flavor	57.70[b]	65.15[a]	4.39
Milk	59.25[b]	70.05[a]	4.64
Butter	51.75	48.90	4.92
Whey	17.45[b]	24.60[a]	4.98
Yogurt	21.35	20.60	4.43
TASTE			
Salty	36.00	39.20	5.57
Sour	28.75[a]	19.40[b]	3.82
Sweet	28.75[a]	19.40[b]	3.06
TEXTURE			
Peelability	65.85[a]	44.45[b]	4.19
Tenderness	78.70[a]	55.35[b]	4.31
Elasticity	58.80[b]	68.65[a]	4.47
Juiciness	69.60[a]	47.20[b]	4.21
Cohesiveness	62.35[b]	72.25[a]	4.01
Chewiness	66.5[b]	91.15[a]	5.04
Harshness	63.20[b]	77.90[a]	4.80

[a,b] Difference between diets ($P<0.05$)

Table 9c. Acceptability of mozzarella cheeses for DRY and FRESH groups.

SAMPLE	DRY	FRESH	LSD
Total acceptability	6.56	6.59	0.38
Aspects	6.51	6.85	0.36
Taste	5.69	5.87	0.40
Texture	6.03	6.02	0.37

FIGURES

Figure 1c. Flow diagram of traditional mozzarella di buffalo Campania (PDO) cheese making

buffalo milk
↓
added natural whey culture (at 10 %)
↓
rennet
↓
curd cutting
↓
maturation of the curd under whey
↓
stretching
↓
shaping in small pieces (20 kg of buffalo mozzarella cheese)
↓
salting
↓
packaging
↓
Sale

Figure 2c. Triangle plot of normalized slopes

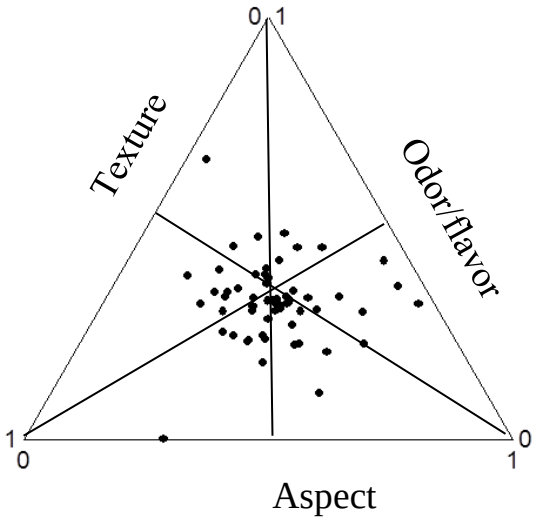

Figure 3c. PCA of FRESH and DRY milks

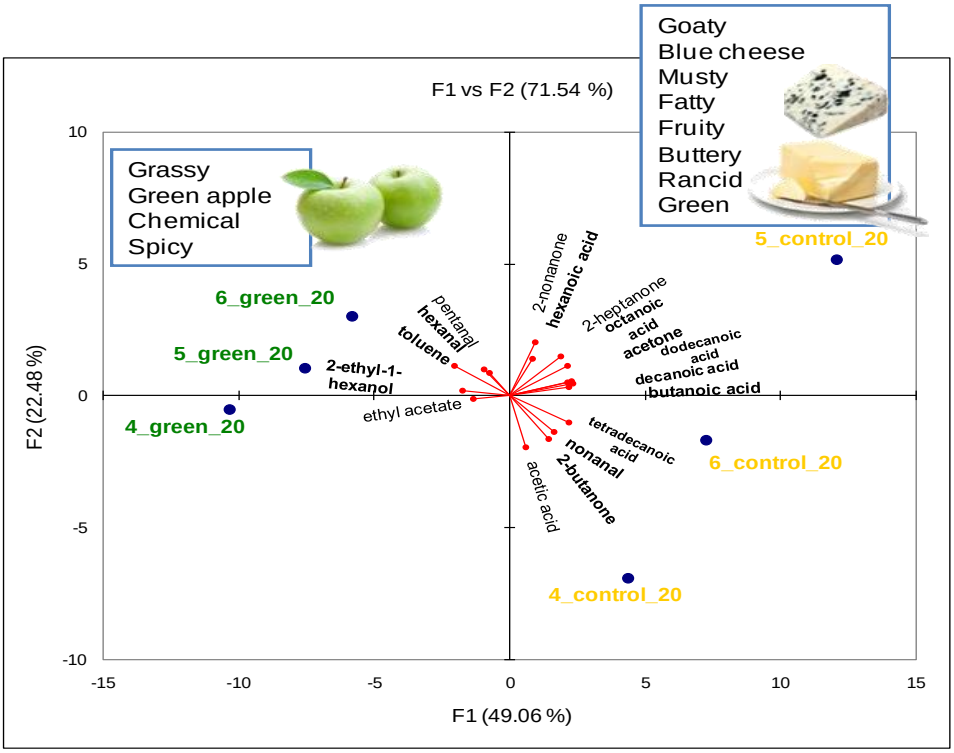

Figure 4c. PCA of FRESH and DRY mozzarella cheeses