

Podolian cattle: reproductive activity, milk and future prospects

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Abstract

In the present study, an original article about Podolian cattle and its milk was performed. In detail, the following factors on reproductive career of Podolian cattle in semi-extensive and extensive rearing were analysed: age of calving, gestation length, calving interval length, abortions percentage, conception and calving period. Chemical and physical parameters (protein, fat and lactose), somatic cell count, lysozyme content and antiradical activity, with ABTS and DPPH assays, of Podolian milk in semi-extensive and extensive rearing were evaluated. Finally, suggestions on future prospects of Podolian milk use were proposed: the preparation of hand soap with 5 percentage of podolian milk and the potential role of this breed for prevention and propagation of fire. The data concerning the reproductive career, milk and environmental role of 677 cows registered in the Herdbook were analysed. The results showed that the 58.56% of the cows manifested a calving interval of 11-14 months. The reproductive activity occurred in spring; over 70% of the cows calved in the period February-April. The chemical-physical aspects of milk are in agreement with literature. It presents an high antioxidant activity values for each group (97.03% and 97.50% for ABTS and 52.09% and 52.60% for DPPH, semiextensive and extensive system

respectively). The consumer test shows that the subjects appreciated the soap containing 5% of Podolian milk for different aspect. The data about environmental role were obtained from Podolian cows reared in fifteen grazing areas were monitored for five years during the summer pasture, when the Podolian cattle graze on green grazing areas located in Basilicata region (South of Italy). Therefore, an efficient management of grazing by Podolian cattle could be an important tool to prevent the fire propagation.

Introduction

The Podolian cattle (*Bos taurus podolicus*) arrived in Italy from the East Asia across the Danube Hungarian plain is well adapted to the harsh environmental conditions of the inland areas of southern Italy (Abruzzo, Basilicata, Calabria, Campania, Molise and Apulia) thanks to its extraordinary ability to survive and reproduce (Felius *et al.*, 2014). This breed presents a lightweight skeletal structure with strong feet and it is very robust and frugal, currently, about 23,000 head are registered in the Herd Book and the animals are reared on more than 600 farms (Maretto *et al.*, 2012; ANABIC, 2014). In past years, this breed was appreciated for its triple attitude (work, meat, milk). Currently, it is instead selected, monitored and evaluated mainly for the production of meat. The Podolian milk is used exclusively for the production of the typical stretched curd cheeses with good quality. This milk is rich in protein (4.06%) and fat (4.87%), contains different bioactive components such as peptides, vitamins (C and E), carotenoids and flavonoids with antioxidant properties (Simos *et al.*, 2011). It is also rich in unsaturated fatty acids (30%), in particular Omega 3 and Omega 6, important in moisturizing functions for the skin (Marsico *et al.*, 1993). The lactation persists about 6-8 months with a total daily production between 5 and 10 liters (Parisi, 1950). The Podolian cows do not fit the mechanical milking, they must be milked by hand simultaneously with the calf feeding, it involves the higher costs and comprehensible difficulties (Procopio *et al.*, 2005). The calving interval is one of the most important parameters to evaluate the productive and reproductive efficiency in a livestock and/or population, but the pedoclimatic conditions, and a high coefficient of inbreeding in populations of poor consistency as Podolian breed, make the analysis of these reproductive characters particularly complex. Studies conducted in farms with extensive system (Giourga *et al.*, 1998) have shown that certain factors, such as diet and photoperiod, can influence the length gestation of certain Spanish genetic types. Several studies (Zicarelli *et al.*, 1989) also showed that the most innovative reproductive technologies (synchronization and embryo transfer) had poor results, and only the best weather conditions and increased food availability may influence, in part, the reproductive activity. The long duration of calving interval and of seasonal reproductive

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activity, limit the desired development and success of Podolian cattle. The enhancement of this model (Musto, 2003), in fact, cannot be tied exclusively to the sale of products that this livestock allows to obtain, but must necessarily be accompanied by the improvement of management. A possible alternative employment and exploitation of the Podolian milk may be to use it as an ingredient for the formulation of natural cosmetic products, being a natural tensor, rich in vitamins, minerals, antioxidants, proteins, enzymes and lipids, the milk well promotes the protection and regeneration of the epidermis (Cosentino *et al.*, 2014). The European market, primarily in Denmark, offers cosmetics based on cow's milk, with emollient and moisturizing characteristics, without paraben, inorganic polymers or other synthetic arising. In particular, milk of animals raised on mountain natural grazing not treated with herbicides and chemical fertilizers, is a good basic constituent for the preparation of natural cosmetics such as milk-soap (Gilbert *et al.*, 2012).

Nowadays, the survival of the Podolian cattle and the possibilities for consolidation of their breeding, are closely linked to the protection of marginal lands and to the preservation livestock biodiversity. The Pan-European Strategy on Biological Diversity has encouraged, in Italy, the establishment of new protected areas and parks for the protection of extensive rearing system that is oriented towards the breeding of native species in order to combat the abandonment of internal areas (Cosentino *et al.*, 2010). This type of breeding, if managed with rational criteria, may exercise specific actions in the habitat conservation: with the containment of some invasive species and poor food value, it allows to maintain a high plant diversity, with the appearance of floristic specimens also particularly valuable, such as *Anacamptis morio*, *Orchis italics*, etc. (Pihl *et al.*, 2001; Freschi *et al.*, 2015). Moreover, grazing animals remove biomass, one of the most important factors for pasture ecosystem management (Leonard *et al.*, 2010). Podolian cattle is well appreciated also for sustainable and ecological management of the available resources.

This article contains the following studies about Podolian cattle conducted in the areas of Basilicata region: i) the seasonality of reproduction and the indices that define the calving interval, and the possible effects of the farming system; ii) chemical-physical characteristics of milk; iii) future employment of milk-cosmetics; iv) environmental role.

Materials and methods

Evaluation of reproduction index

This study was conducted analysing data from 24 farms located in different areas (Val d'Agri, Camastra, Alto and Medio Basento) of Basilicata, South of Italy. The farms were selected for the same number of cows, and were divided for rearing system: extensive and semi-extensive. In the extensive system, the grazing was throughout the year without shelter; in the semi-extensive system, the grazing was during the year, with shelter only for the colder season. The data concerning the reproductive career of 677 cows registered in the Herdbook were analysed. For each cow, the dates and the age of the calving have been detected, defining the gestation length, the calving interval and the conception and calving period. In both farming systems, the natural service is with a sex ratio of 1 bull per 30 cows in order to get the calvings in a limited period.

Chemical and physical analysis of Podolian milk

Bulk cow milk was taken on the same day from 4 farms (2 for extensive system, 2 for semi-extensive system) that used mechanical milking. The farms are situated at about 700 m above sea level in National Park of Appennino Lucano, Basilicata. After collection, milk aliquots were immediately refrigerated at 4°C and transported to the laboratory for analytical determinations. On milk samples we measured pH (HI931410, Hanna Instruments, Padova, Italy), protein, fat, and lactose content according to the International Dairy Federation Standard (ISO, 2013) by Milkoscan FT 6000 FT 6000 (Foss Electric, Hillerød, Denmark). Somatic cells count, expressed as SCS ($\log_{10} n \times 1000/\text{mL}$) (ISO 13366-2:2006) we determined using a Fossomatic 5000 (Foss Electric A/S). Moreover, we enumerated bacteria total count. All determinations were carried out in triplicate.

Lysozyme quantity of Podolian milk was determined by HPLC fractionation using a reversed-phase column. Sample milk preparation, column equilibration and elution were performed according to Cosentino *et al.* (2016). The chromatographic separations were run on a Synergi MAX-RP 80 Å column (150×4.6 mm, 4 µm particle size) from Phenomenex (Torrance, CA, USA) with a MAX-RP guard column (4×2 mm id). Injection volume was 20 µL and flow rate was 0.8 mL/min. The mobile phase consisted of a gradient of water (A) and acetonitrile (B) both containing 0.1% trifluoroacetic acid (v/v). Eluting conditions are: 0 min 80% A and 20% B; 9 min 60% A and 40% B; 15 min. 60% A and 40% B; 20 min. 80% A and 20% B. Detection was carried out by fluorescence detector (Jasco FP-2020 Plus-Intelligent-fluorescence detector) set at 280 nm excitation and 350 nm emission. Calibration curves were acquired with known amounts of HEW lysozyme in the concentration range of 5 to 100 mg/L.

Antiradical activity of Podolian milk was evaluated by using both DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-acid)) assays (Cosentino *et al.*, 2015). Both tests were carried out in triplicate.

DPPH assay: The stock radical solution of DPPH was prepared by dissolving 20 mg of DPPH in 15 mL of ethanol. After 1 min of agitation with Vortex, 1 mL of stock DPPH solution was diluted in ethanol (1:30). 50 µL of milk were added to 950 µL of DPPH solution and incubated into the darkness for 30 min at room temperature. After centrifuging (5 min, 8000 rpm), absorbance was measured at 515 nm against the reference solvent (ethanol) by using spectrophotometer UV-Vis (LKB Biochrom 4050 Ultrospec II).

ABTS assay: The stock solution of the ABTS radical was prepared by dissolving 38 mg of (ABTS) in 10 mL of an aqueous sodium persulphate solution (2.45 mM). The mixture was dark stored for 12-16 h. For the analysis, 1 ml of stock ABTS^{•+} solution was diluted in ethanol (1:30). 20 µL of milk sample was added to 980 µL of ABTS^{•+} solution. Milk samples were reacted with ABTS^{•+} working solution for 2 h in incubation into the darkness at room temperature. After centrifuging (5 min, 8000 rpm), absorbance was measured at 734 nm against the reference solvent (ethanol). The solutions were prepared fresh for the analysis.

Antioxidant activity of cow milk was evaluated through the Radical Scavenging Activity (RSA%) utilizing the following formula: Radical Scavenging Activity (RSA%) = $(1 - A_i / A_0) \times 100\%$, where A_i is the absorbance of sample and A_0 is the absorbance of colorimetric radical substance.

Preparation and efficacy test of hand soap

Hand soap supplemented with 5% percentage of Podolian milk was prepared by specialized and certified laboratory. The surfac-

tants used are derived from cornstarch and coconut oil. The abrasive action is carried out by plant micro-granules obtained by crushing the shell of hazelnut and almond; the fragrance is given by the essential oils of orange and rosemary, able to eliminate bad odours. The Podolian milk and vegetable Glycerine help to restore the hydro-lipid barrier of the skin. The basic formula of liquid soap is the following according to International Nomenclature of Cosmetic Ingredients (INCI): aqua, sodium lauroyl sarcosinate, cocamidopropyl betaine, acrylates copolymer, lauryl glucoside, lac, citrus autantium dulcis oil, prunus amygdalus dulcis shell and corylus avellana shell, glycerin, benzyl alcohol, rosmarinus officinalis oil, sodium benzoate.

The soap was tested by 8 women and 7 men regular consumers of liquid soap recruited from our University Campus. Their age was in the range 22 to 54 years. For evaluating the effectiveness in removing dirt, in relation to the target professional profiles identified in our previous study for willingness to buy (Cosentino *et al.*, 2014), soiling products were employed, such as engine oil, grease workshop, plaster paste, universal soil, extra virgin olive oil, margarine, dough for bread, charcoal, minced meat. The soiling of the hands, the movements and the operations of washing, including the amount of soap used was standardized. During efficacy test, the individual washes are controlled for the amount of product used, the times and the movements of cleaning.

Environmental role of Podolian cattle

In fifteen grazing areas located in 14 different municipalities of Basilicata, the occurrences of fire were monitored for 5 years, 2010-2014 (Pecora *et al.*, 2015). In these areas only Podolian cattle grazed (Figure 1). The study areas were drawn by using open source GIS software, the burned areas were recorded by using GPS (Garmin Montana 60T) and classified in two classes: wooded burned area (WBA) and no-wood burned area (NWBA). The grazing and burned areas were analyzed by GIS technique. The extension (ha) of fire damage was measured within and in the surroundings of each grazing areas (no-grazing area) by overlay.

For livestock production system, data of consistency and of live weight on Podolian cattle kept on each grazing area were obtained in personal interviews with 15 farm operators. The number of animals was used to calculate the number of Livestock Unit, which, according to the European official regulation (Commission



Figure 1. Podolian cows on grazing in the national park of Appenino Lucano.

Regulation - EC n. 1200/2009), it is defined as follows: 0.0 LU for calves younger than 6 months, 0.6 LU for cattle between 6 months and 2 years, and 1.0 LU for cattle older than 2 years. For each area, the stocking density (SD) was calculate by dividing the number of LU of each area by the extension (ha) of the same area.

For pasture utilization, the potential feed intake of Podolian cattle in each area was estimated according to Grenet *et al.* (1987): dry matter intake for adult cattle is 14 g DM/kg LW (DM dry matter, LW live weight). While the live average weight was 605 kg, mean of data reported on farm registers. The removal of potential biomass by Podolian cattle was estimated by multiplying the feed intake calculated in each area for 120, summer season characterized by highest incidence of fire, as well as the peak of the dry and hot temperatures combined with the lowest rainfall (Pecora *et al.*, 2015).

Results and discussion

Evaluation of reproduction index

The results showed that the average gestation length was 281 days. Similar values were reported by Caballero de la Calle (2003) in a study about the breed *de Lidia*. The neonatal mortality rate was 3.15%, while spontaneous abortions were 1.95% (Figure 2). There were not marked differences between the two rearing systems, but only slight improvements in reproductive indices in semi-extensive rearing due, probably, to the shelter in cold season and to the dietary supplementation. The cases of neonatal mortality (2.7 vs 3.6%) and of spontaneous abortion (1.8 vs 2.1%) were lower in semi-extensive than extensive rearing. The calving concentration in the period from February to April was higher in semi-extensive than in the extensive rearing (72.51 vs 70.66%; Figure 2). According to Panella *et al.* (1995) and Montemurro (1996), the facility in calving performing, even in harsh environmental conditions, is due both to the pelvis anatomy that to the functional gymnastics caused by the intensive grazing intrinsic in both rearing systems. The reproductive activity was higher in the late spring and early summer, with the highest concentration of conceptions in May for both considered systems. The calving interval was between 11 and 14 months in 58.56% of the cows, it increases up

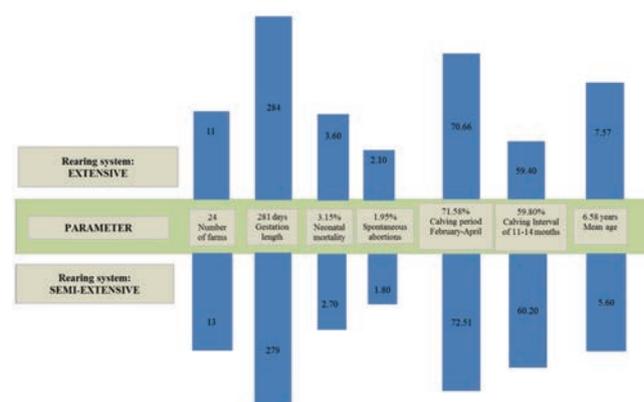


Figure 2. Comparison among the parameters studied in the two rearing systems.

to 24 months or more in the 38.24% and is less than 11 months in 3.2% (Figure 3). In our study the percentage of cows with a calving interval within 14 months was lower than that one (70-80%) reported by Montemurro (1996) in a similar production area. In the semi-extensive rearing system, a greater percentage of cows was observed with a calving interval between 11 and 14 months than the extensive rearing (60.20% vs 59.40%). The improvement could be due to the reduction of the inbreeding coefficient, as the significant increase in animals registered in the Herdbook (+26.51%) (ANABIC, 2014) during the last decade and the subsequent reduction of mating among relatives. In the semi-extensive rearing system, in addition, the improvement of reproductive parameters may also be due to a greater quality and availability of forage that, by reducing the energy deficit, reduces the marked seasonality of reproductive characters typical of the Italian beef cattle (Panella *et al.*, 1995). The data show also a cyclical average duration in calving interval of total observed cattle (Figure 3), with two peaks: the one at 12th month (20.43%) and a second one at 24th month (6.00%). The calving period is concentrated from January to June for 80% of total observed cattle, February March and April are the months with the highest concentration of calving (71.05%), only 25.2% of the calves occurs in mid-summer (July-August) (Figure 4). The data reported are difficult to compare with other studies and other Italian rustic breeds (Maremmiana, Romagnola, Marchigiana, Chianina), because the analyzed parameters depend on environmental factors and on observed areas (Sargentini *et al.*, 2009).

The average age of the observed cows in the two rearing systems is of 6.58 years, with a minimum of 4 and maximum of 13 years. The longevity is a distinctive feature of farming systems,

especially in farms with extensive system, results in a low culling index.

Chemical and physical analysis of Podolian milk

Several experimental studies have shown that milk from cows fed on pasture has particular characteristics compared to the milk of cows fed indoors. The grazing influences the most important chemical and biological parameters defining the quality of milk (fat, protein, urea, somatic cells).

In Table 1 were reported the chemical composition of Podolian

Table 1. Chemical-physical aspects of Podolian milk for each rearing system.

Parameters	Semiextensive		Extensive	
	Mean	± S.D.	Mean	± S.D.
pH	6.65	± 0.05	6.68	± 0.06
Protein, g/100 g	3.60	± 0.06	3.65	± 0.05
Fat, g/100 g	3.96	± 0.05	4.00	± 0.05
Lactose, g/100 g	5.11	± 0.04	5.20	± 0.06
Lysozyme, mg/L	0.25	± 0.04	0.26	± 0.05
RSA%, DPPH	52.09	± 3.40	52.60	± 3.85
RSA%, ABTS	97.03	± 1.06	97.50	± 1.20
SCC, cell/mL	76,600	± 500	78,900	± 700
Bacterial count, CFU/mL	340,000	± 1300	365,000 ±	1200

S.D., standard deviation.

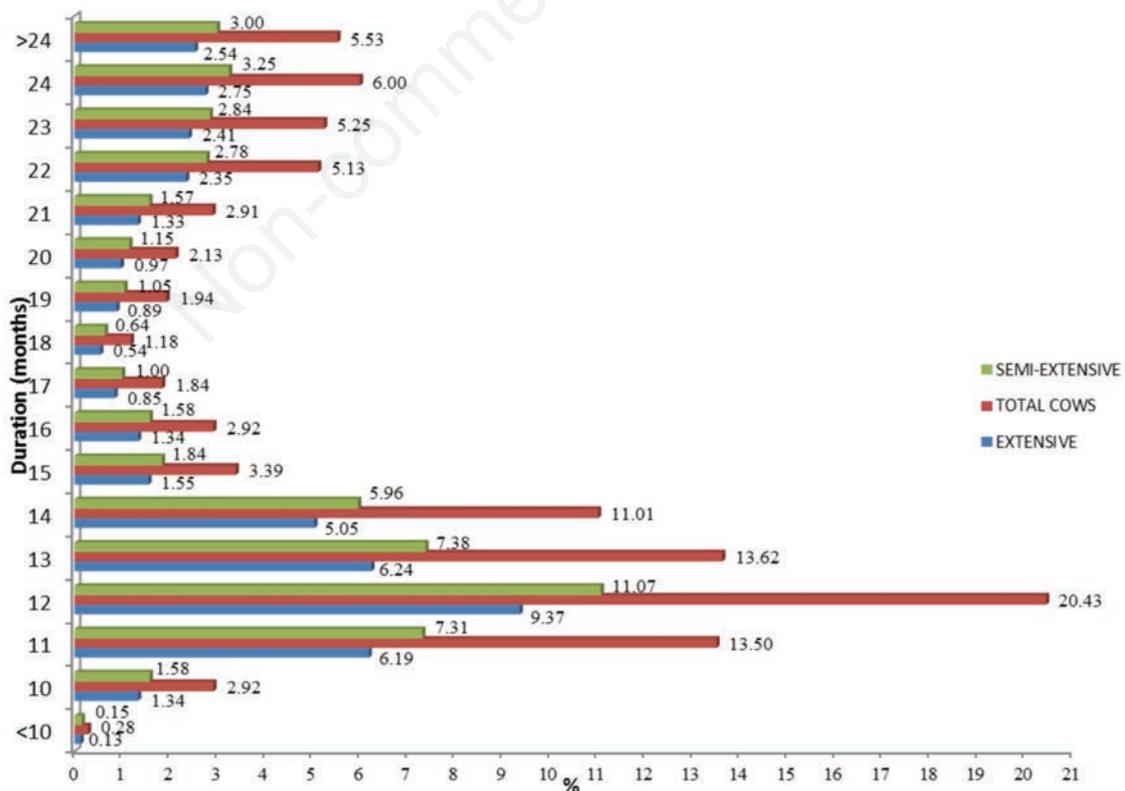


Figure 3. Calving interval in each rearing system and in total observed Podolian cows.

milk rearing in semiextensive and extensive system. There were not marked differences between the two rearing systems. The antioxidant activity of Podolan milk is very high for both assay (97.03% and 97.50% for ABTS and 52.09% and 52.60% for DPPH, semiextensive and extensive system respectively). Podolian milk shows a content of lysozyme in trace in agreement of literature. In cow milk lysozyme content was generally <0.6 ppm, according to Claeys *et al.* (2014) and varies depending on the lactation period, at the beginning it is higher (Dimitrov *et al.*, 2009).

Efficacy test of hand soap

The aim of this preliminary study was to evaluate the effect of an alternative ingredient on some sensory aspects of skin in fifteen volunteers. The results of our study show that the subjects appreciated the soap containing 5% of Podolian milk for different aspect. Results showed maximum preference for following parameters: the *exfoliating power*, the *cleaning power*, the *skin hydration* and the perfume that the soap leaves on the hands. Cow milk preparations (face and body creams, cleansing milk, and tonic) are the most known by consumers. Dairy products are good remedies for imperfect skin, Vitamins B, A and E contained in the milk have a regulating and regenerative effect. Proteins and other components have a strong absorption capacity and water retention, encouraging a high degree of hydration of the skin, and preventing the degradation of the epidermal cells (Temmuujin *et al.*, 2006). Cotte (1991) put in evidence an increased elasticity and a calming effect of the skin of a cream containing cow milk. These results

confirm that podolian milk could be a cosmetic component suitable for all skin types thanks to its balancing skin's moisture.

Environmental role by Podolian cattle

GIS analysis showed that fire affected only 4 areas of 14 municipalities of Basilicata: 1, 8, 12 and 15 (Figure 5).

Results showed in the Area 1 (Abriola/Pignola municipalities), the burned area had an extension of 120 ha in 2011, and 3.35 ha in 2012. In the grazing area (2011), 25 ha of WBA and 24 ha of NWBA were recorded, with a percentage incidence on the total grazing area of 4.7% and 4.4%, respectively. In the no-grazing area, there were 35 ha of WBA and 41 ha of NWBA, with a percentage incidence on the total no-grazing area (buffer area) 7.5% and 8.8%, respectively. In the 2012, 3.35 ha of NWBA were recorded in the grazing area (0.6% of grazing area). In 2012 in the Area 8 (Ferrandina municipality), there were only 5 ha of WBA in the grazing area (1.5% of total area); in the Area 12 (Pescopagano municipality), the burned area had an extension of 2 ha of NWBA (0.3% of total of no-grazing area). During 2011, in the Area 15, there were 1 ha of WBA (0.1% of total grazing area), 7 ha of WBA and 3.5 of NWBA (1.8 and 0.9% of total of no-grazing area, respectively). These results show that in the grazing areas, the incidence of fire propagation was very low.

The potential effect of removal biomass by Podolian cattle may be useful as a tool to prevent the fire propagation. In fact, these breed showed, during the time, a number of positive environmental effects, such as increased climate stability, improved soil functionality, water quality and footprint and preservation from

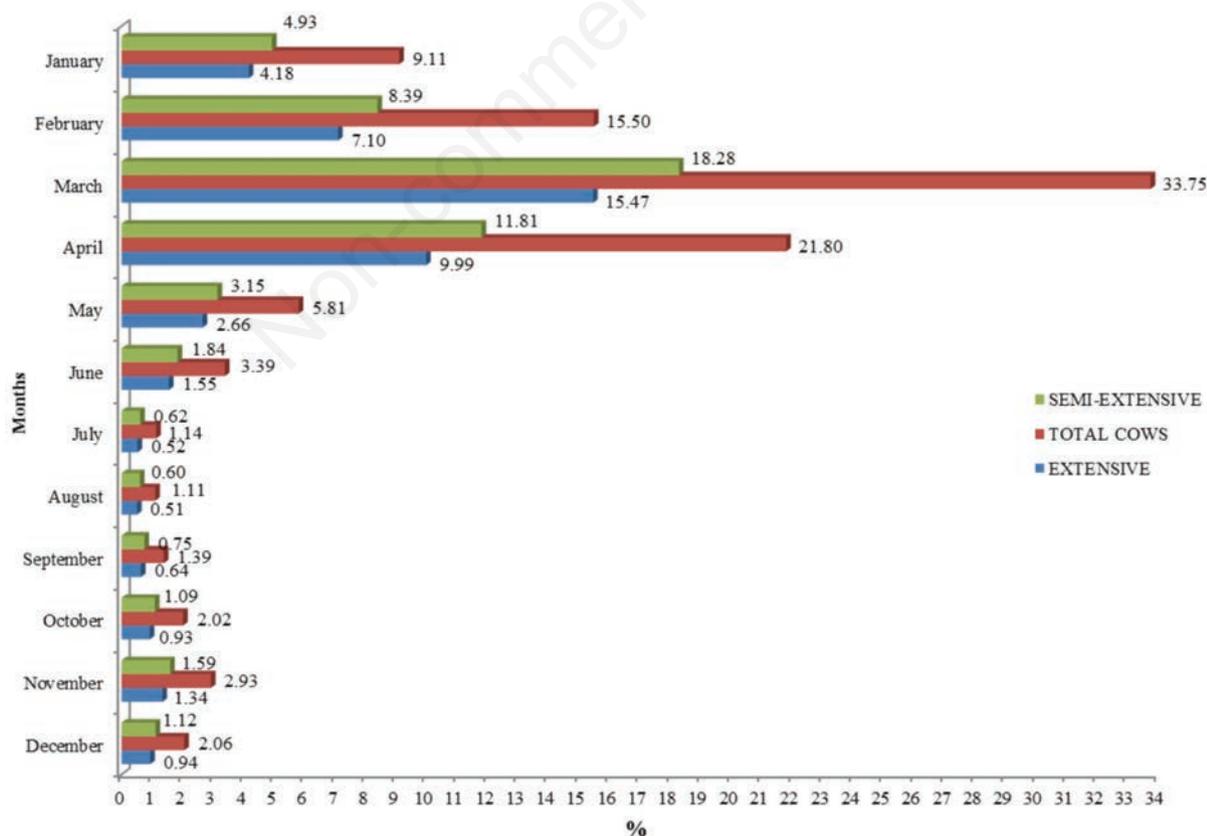


Figure 4. Calving frequency in each rearing system and in total observed Podolian cows.

fires (Freschi *et al.*, 2015).

In Figure 6 are reported the main characteristics of Podolian cattle reared in the areas. Concerning stocking density (SD), in two grazing areas (Area 6 and 9) the parameter was higher than in the other grazing areas. This was due to both small grazing surface and high consistency of Podolian cattle. SD resulted to be below the threshold values laid down in Nitrates Directive (91/676/CEE). However, it is recommended to keep a low livestock intensity in order to not create overgrazing, which in turn may lead to soil compaction by trampling, reduction of water infiltration, and increased surface run-off and erosion (Strand *et al.*, 2014; Freschi *et al.*,

2015). Moreover, the value of dry matter intake for grazing time (DM) was the highest in 2013 due to substantial cattle turnover. This estimation allowed understanding how the Podolian cattle browsing may be an important tool to reduce the fuel in order to create a horizontal and/or vertical fuel break. In fact, the Podolian cattle may be an interesting tool for fuel reduction, just like goat (Lovreglio *et al.*, 2014). Obviously, this goal should be achieved through appropriate measures, such as the use of metallic or electrified fence in order to maintain an appropriate stocking density, for browsing both the available foliage and twigs from all woody plants and all herbaceous vegetation.

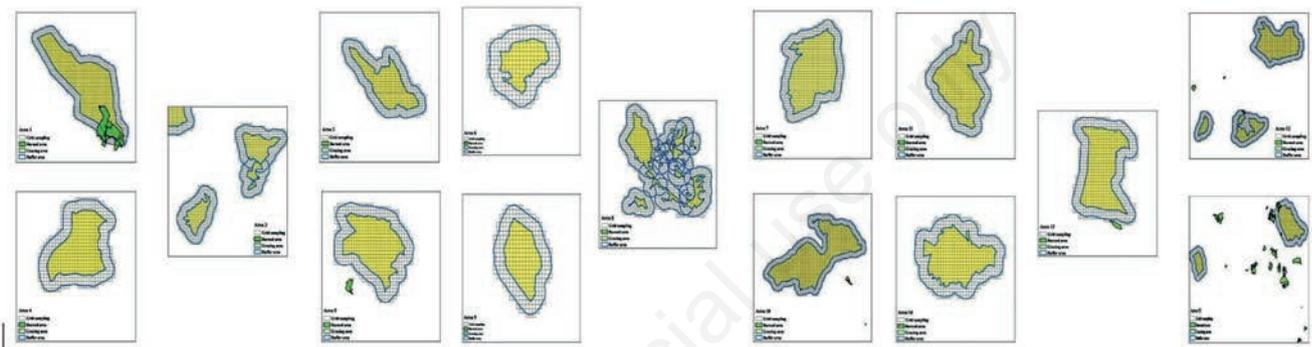


Figura 5. Grid sampling mapping: areas 1 to 15.

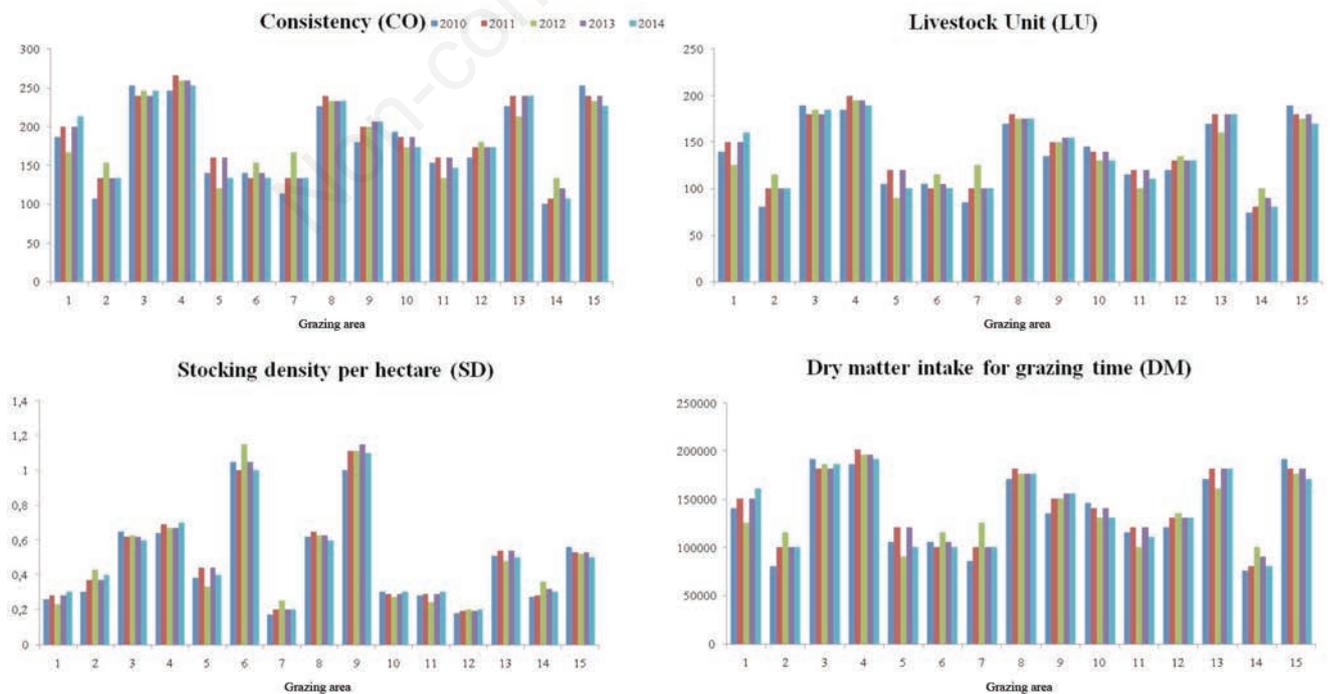


Figure 6. Feed intake estimation of Podolian cattle from 2010 to 2014 - Potential feed intake of Podolian cattle.

Conclusions

This study on the Podolian cattle from the southern Apennines confirms that environmental factors affect the reproductive features. The Podolian cattle is, in fact, characterized by a high concentration of births in spring, when the natural pastures and meadows are in maximum productivity, with direct effects on the physiological state of the cows and on the milk production. This feature allows the rational use of spontaneous forage production. Despite the high containment of the rearing costs, the trade in veal calves depends on the age and live weight during the year, with a maximum between August and December (subjects 15-18 months). Moreover, the calving interval is quite high and shows a periodic trend with maximum at 12th and 24th months. Thus, the preservation of this cattle and the biodiversity conservation *in situ* native, involves improving the performance and control of inbreeding. Furthermore, we reported for the first time in literature, the chemical-physical aspects of podolian milk about fat, lactose, protein (in particular lysozyme) contents and about pH, bacterial count and antioxidant activity values. Due to the high antioxidant activity of this milk, the Podolian milk should be employed in cosmetics. For the revaluation of Podolian milk, we chose to use it as raw material for a skincare product that was positively evaluated by 15 testers. The placing of a new soap hands in the cosmetic industry would allow to small breeders of Podolian cattle to improve their incomes. The use of Podolian milk to realize a hand soap with exfoliating and cleaning power but delicate and emollient, it aims to capture the large target of consumers of effective products but natural and respectful of the skin.

Concerning grazing, the prescribed grazing with Podolian cattle can reduce the fuel load of shrublands, grassland in the short term by partially reducing woody fuels. Moreover, livestock grazing may reduce fire ignition potential and spread by removing live and dead herbaceous vegetation and accelerating litter decay through trampling. However, the stocking density should not excessive in order to manage the pastureland and to reduce the fire propagation. In conclusion, exploiting effectively the local resources, this breed can be regarded as a genetic resource to improve the economy in the marginal areas in a sustainable and environmentally friendly way.

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