UNIVERSITÀ
DI PARMA

DIPARTIMENTO DI SCIENZE MEDICO VETERINARIE

Corso di Laurea Magistrale in

Produzioni Animali Innovative e Sostenibili

GENETIC AND PHENOTYPIC VARIABILITY IN A LOCAL FARM OF DAIRY GOATS WITH POTENTIAL PRACTICAL APPLICATION

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ANNO ACCADEMICO 2022/2023

SUMMARY

ABSTRACT

In the Italian alpine and hilly regions, goat farming has been a noteworthy sector of the livestock industry, closely linked with the production of cheese and meat. The consumption of goat meat and cheese has rapidly increased in recent years in our country, underscoring the importance of delving into and understanding all aspects of this type of farming. This thesis aims to analyse the 'La Dinara' dairy Camosciata delle Alpi goat breed farm located in Terenzo in the Parma Apennines (Italy) from both a genetic and phenotypic point of view, analysing the pedigree and individual milk samples of the animals present in the farm.

From a genetic variability point of view, in this thesis a dataset was created containing the pedigree of all the animals present on the farm from its creation till today. A total of 224 animals were recorded into the dataset which was then analysed using the ENDOG v4.8 software thanks to which the Average Relatedness (AR), Inbreeding (F) and the rate of inbreeding per generation (ΔF) data were analysed.

As regards the phenotypic analysis, the milk of the lactating goats present on the farm was analysed. A total of 28 samples were analysed, divided into two samplings: during the first sampling the goats lived in indoor conditions and in the second sampling in the pasture.

The samples were analysed at the MilCa laboratory in the University of Parma.

Analyses were carried out on the samples for the composition, hygienic quality traits, for the cheese-making traits and milk coagulation properties.

The data obtained from milk analyses were subsequently compared to additional information derived from samples of the same goat breed sourced from three farms situated in Lombardy belonging to the BIO4VERBA project.

A statistical model was therefore developed including the effects of region, housing condition, farm and the interaction between the region and the housing condition.

The results obtained from both genetic and phenotypic analyses highlighted good management in terms of mating and animal welfare.

Concerning genetic variability, the observation of zero inbreeding (F) within the reproductive population (RP) is noteworthy in the ‘La Dinara’ farm. The fact that there's no inbreeding suggests that there is a lot of genetic variety and indicates good herd management. Also from the milk analysis, the obtained results exhibit interesting characteristics, both regarding milk composition and cheese-making traits.

However, the company can implement some measures to ensure that there is no loss of genetic variability over time and to further improve the characteristics of the milk produced.

1. INTRODUCTION

In contemporary times, the concept of sustainability is a crucial concern within production systems, particularly in the context of animal production, and frequently takes centre stage in various discussions. Initially, systems were deemed unsustainable either when a crucial resource was depleted to the extent that it became unavailable to the system or when a product of the system accumulated to a degree that hindered its proper functioning (Tivy , et al., 1982). Nowadays, the meaning of the term is much wider and should take into account the needs of the present and the future (Stavins , et al., 2003). As regards animal production, a very efficient and profitable systems is the semi-intensive silvopastoral production of meat or milk, since they are good for biodiversity and animal welfare, and reduce greenhouse gas and pollution per unit of production (Broom, et al., 2013).

Therefore, the development of small non-industrial and semi-intensive breeding companies could represent a good compromise between both animal and economical sustainability.

Semi-intensive breeding provides shelter in the stable for the night in the summer and spring months and the remaining part of the day the flock grazes; while in the colder periods of the year, in autumn and winter, the shelter in the stable is permanent. This kind of breeding supports the economic component while respecting natural requirements, animal welfare, and environmental sustainability.

The 'La Dinara' farm examined by this study is a family-run business, dealing with semi-intensive dairy goat farming located in Terenzo (PR, Italy) in the rural context of the Parma Apennines. During my internship on this farm in May, June, and July of 2023, I chose to undertake this study.

When the farm was first established in 2015, the herd consisted of 6 females and 1 male all belonging to the Camosciata delle Alpi goat breed. A dairy factory was built next to the stables in the summer of 2015 so that milk could be turned into cheese.

There are currently 29 Camosciata delle Alpi goats on the farm, 2 Cabannina cattle used for meat production, 2 Maremmano-Abruzzese Shepherd dogs for the protection of the flock and a Border Collie for guiding of the flock.

Figure 1- Grazing animals at the 'La Dinara' farm.

The farm includes 10 hectares of land used as pasture and for the production of hay and straw, approximately one hectare of land is used for the production of wheat intended to the production of flour.

In addition to the production of goat's cheeses, the company has also developed other ideas such as making flour and pasta from its own wheat and cosmetics based on goat colostrum. The company occasionally offers goat and cow meat as well.

They sell all the products through local "Farmer's Markets" and to typical restaurants in the area.

1.1 GENETIC VARIABILITY

Until the development of this study, the owners of the company kept a paper genealogical register from which they based the mating.

To enhance the mating program through the utilization of ENDOG V.4.8 software, we opted to generate a dataset encompassing all the animals from the farm's creation to the present. Inbreeding and average relatedness were the key variables that were taken into consideration and examined using ENDOG v.4.8.

The data sets were analysed taking pedigree completeness (CGE - complete generation equivalent) into account since pedigree completeness is key when evaluating the genetic diversity based on genealogical data.

1.1.1 Inbreeding and average relatedness

Inbreeding (F) can be defined as the probability that two alleles at any locus are identical by descend and occurs when related individuals mate (Malecot, 1948). In other words, from a population point of view, inbreeding is the consequence of mating between two individuals who are more related to each other than the average relatedness in population, which results in reducing in fitness of progenies and genetic variability in populations.

Despite the effectiveness of inbreeding in evolutionary genetics, animal and plant breeding to fix favourable alleles, it is often used improperly and might cause inbreeding depression (Templeton, et al., 1994). Consequence of inbreeding is genotype frequencies changes though increasing homozygosity by expensing of heterozygosity (Charlesworth, et al., 2009). This leads to the redistribution of the genetic variations within and between populations, a reduction in performance traits related to fitness and reveal of homozygous recessive defects. Moreover, it results in genetic drift, interrupts Hardy–Weinberg equilibrium and change in effective population size. Inbreeding depression is a harmful phenomenon in livestock which is outcome of inbreeding (Nostrati, 2017).

The awareness of the detrimental effects of increased homozygosity due to inbreeding prompted the development of actions that aim to minimize accumulation of inbreeding over time (ΔF) (Makanjuola, et al., 2019).

Another important aspect to evaluate is the average relatedness coefficient (AR). When evaluating the AR of a founder animal indicates its genetic contribution to the population. Hence, AR can be used as an alternative or complement to the inbreeding coefficient, to predict the inbreeding of a population in the long term, since it takes into account the percentage of full pedigree originated from a founder.

Average relatedness in genetics refers to the average degree of genetic similarity between individuals within a population. It is a measure of the average proportion of genes that two individuals share due to common ancestry. Relatedness is key to understand the genetic basis of traits, the dynamics of populations, and the evolutionary processes that shape genetic diversity within a population.

One common way to quantify average relatedness is through coefficients of relatedness (AR), which provide a numerical value that represents the proportion of genes shared between two individuals. The coefficient of relatedness ranges from 0 to 1, where 0 indicates no genetic relationship (unrelated), and 1 indicates complete genetic identity (e.g., identical twins). Average relatedness can be influenced by factors such as the structure of the population, patterns of mating, and historical events like population bottlenecks or migrations. Thus, the AR can then be interpreted as the representation of the animal in the whole pedigree.

The advantages of using AR in a population are:

- The AR of a founder indicates the percentage in which this founder influenced the whole population;
- AR can be used as an index to maintain the initial genetic stock selecting as breeding animals those with the lowest AR value;
- AR, as an alternative or complement to F, can be used to predict the long-term inbreeding of a population because it takes into account the percentage of the complete pedigree originated from a given founder at population level. In addition, AR can be used to compute the effective size of the founder population as the inverse of the sum of the square AR coefficients across founder animals (Gutierrez, et al., 2005).

Genetic diversity is a key factor for both adaptation and response to selection. The loss of genetic diversity not only causes a decrease in individual fitness, but it also has a dramatic negative effect on the population lifespan in the long term. Inbreeding generally reduces performance, health and fertility at individual level (Hill, et al., 2004); (Sairanen, et al.,

2009). To prevent the negative effects of the loss of genetic diversity, the FAO sets a maximum inbreeding rate of 1% per generation (Scherf, 2000).

Inbreeding coefficients and average relationships over time have been used to evaluate how genetic diversity evolved from the creation of the flock till today (Hill, et al., 2004). A limited number of individuals can radically affect the rate of genetic diversity, therefore in the following thesis it was decided to study these genetic parameters to highlight a possible decline in genetic diversity given by the low number of individuals in the evaluated farm.

1.2 MILK ANALYSIS

1.2.1 Milk quality traits

The composition and quality of milk depend on many factors such as diet, breed, season, feeding, management, environmental conditions, locality, and health status of the udder.

Goats' milk has some particular properties that confer technological advantages in comparison to cow's milk, such as a smaller size of fat globules, which provides a smoother texture in derived products, lower amounts of alpha1-casein, resulting in softer gel products, a higher water holding capacity and a lower viscosity (Alok Kumar, et al., 2016).

The digestibility of goat milk can be attributed to its casein curd which is both softer and smaller than that produced by cow milk, this makes it more easily digestible and accepted by the human digestive system (Park, et al., 2007).

The mean values of goat milk composition are 3.3% protein,4.0% fat, 4.3% lactose, 0.8% ash and 12.6% total solids (Hammam, et al., 2022).

The milk of different animals has the same essential components event if in different percentage as shown in *Figure 2.*

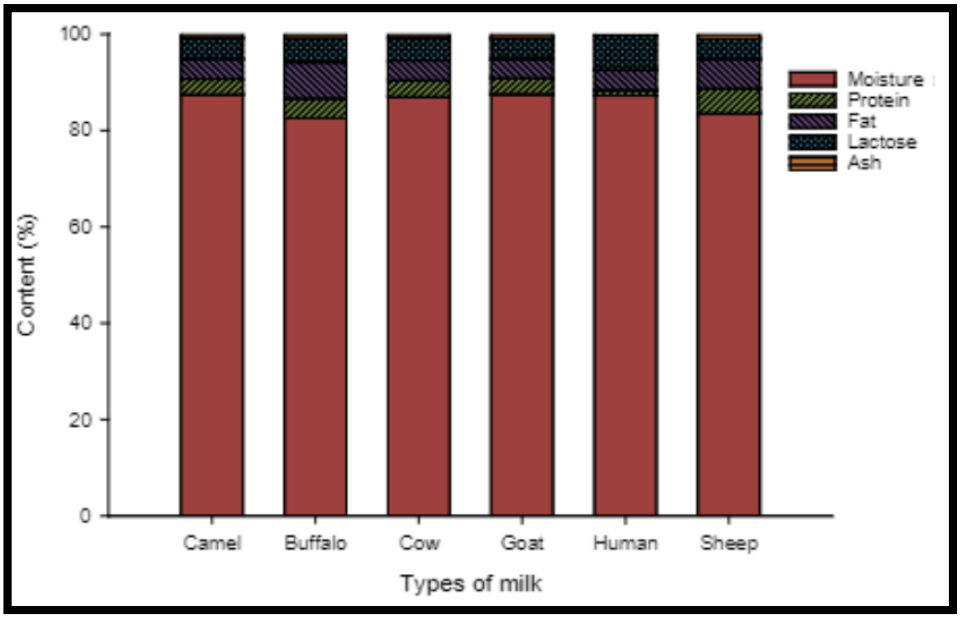

Figure 2 - Mean composition of goat, cow, camel, buffalo and human milk (Soliman, 2005); (Lima, et al., 2018).

The milk quality is not only influenced by its composition but also by the quantity of somatic cells (SCC) and differential somatic cells (DSCC).

The combined use of somatic cell count (SCC) and differential somatic cell count (DSCC), which is the ratio of neutrophils plus lymphocytes to total milk SCC, represents a novel approach to define cow's udder health status, as it allows to identify healthy animals (those with low SCC and DSCC), cows susceptible to mastitis (those where an immune response has begun, so that there is an increase of neutrophils, i.e. DSCC, but not yet of total SCC), animals with a mastitic event in progress (those with high SCC and DSCC) and animals with possible chronic inflammation (those with high SCC and low DSCC, as macrophages prevail) (Bobbo, et al., 2020). Although this novel method has been efficiently used in dairy cattle, it is still not yet implemented in dairy goats. Nevertheless, both SCC and DSCC can be used as indicator of udder health in goats as well.

1.2.2 Milk yield traits

World production of goat milk ranks third, below cow and sheep milk, and is mainly used to produce cheese (FAOSTAT, 2021). The percentage ratio between milk processed and cheese manufactured (%CY) is considered one of the most important attributes of milk affecting the profitability of dairy farmers (Emmons, et al., 1993). Cheese yield relies first on the fat and protein (in particular casein) content of milk, and also on the technological properties of processed milk (Law, et al., 2010); these characteristics can influence the proportion of individual milk components recovered in the curd (%REC) or lost in the whey, directly related to the overall efficiency of cheesemaking process (Banks, 2007).

The increasing demand for goat cheeses in recent decades, coupled with an increase of milk price, has stimulated new interest in the cheesemaking ability of goat milk and formulae predicting cheese yield on the basis of milk components were proposed (Zeng, et al., 2007). The suitability of lactodynamography for testing individual goats milk samples relies on the small volume of milk and the possibility to test several samples in a short period. Traditionally, lactodynamography did not provide direct measurement of %CY and %REC traits, but only reproduces first steps of the cheesemaking process (i.e., rennet addition, milk coagulation, curd firming). However, recent modifications of the analysis procedures proposed by Cipolat-Gotet et al. (2016) (*Figure 3*) permitted the assessment of the phases during which the obtained small curds are cut, heated, and drained (Vacca , et al., 2018).

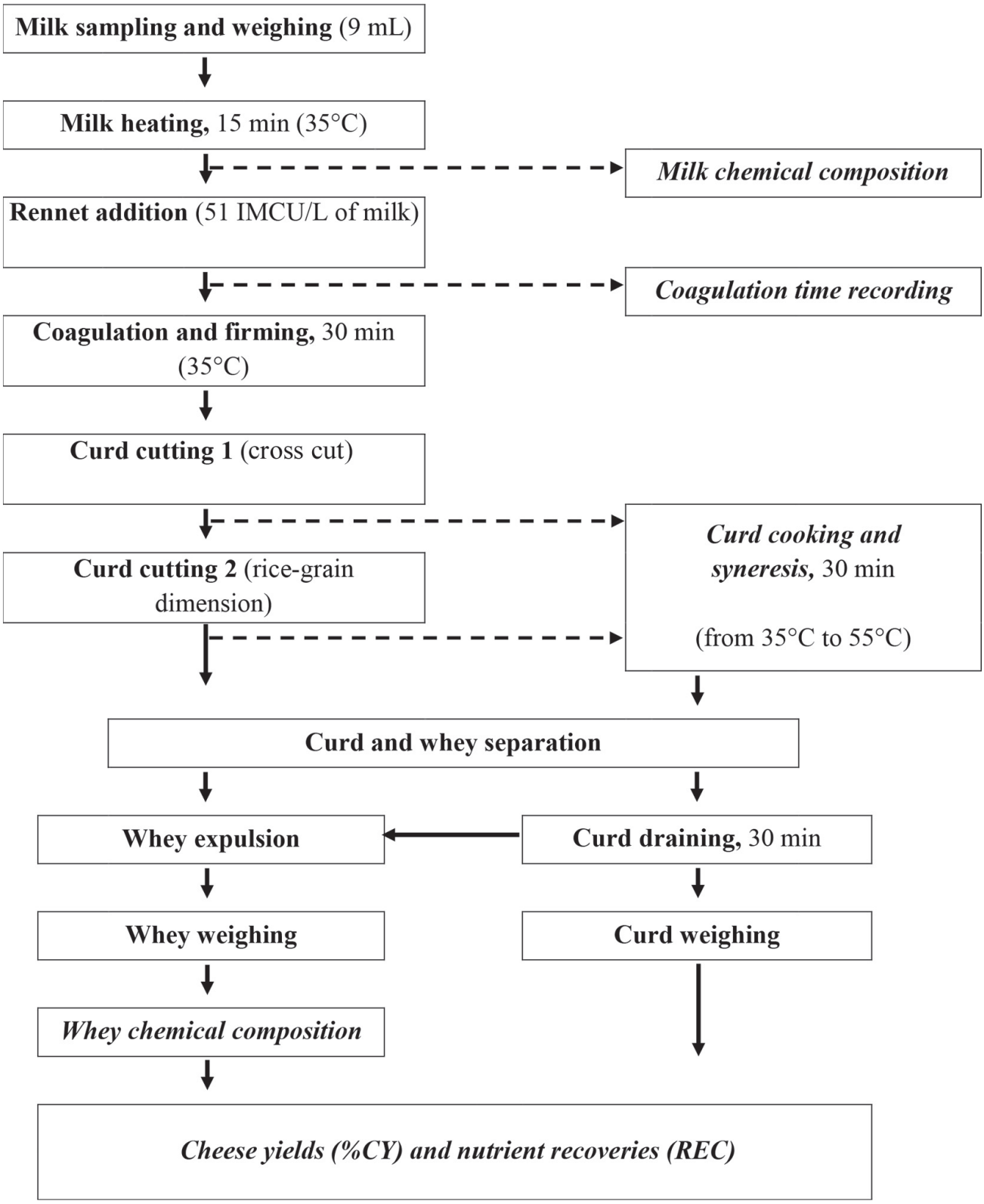

Figure 3 - Flowchart for the 9-MilCA (9-mL milk cheesemaking assessment) method. IMCU = international milk clotting unit. (Cipolat - Gotet , et al., 2016).

1.2.3 Milk coagulation properties

Milk coagulation property (MCP) is an important characteristic and a basic requirement of milk for cheese production (Summer, et al., 2022).

Commonly, MCP is measured as a combination of:

- Rennet coagulation time (RCT, min), obtained by measuring the interval from time zero (the moment of addition of rennet to milk) to the time at which the baseline begins to widen;
- The time to a curd firm ness (CF) of 20 mm (k20, min), which is the interval from the start of gel formation (RCT) to the time at which the oscillation width becomes 20 mm;
- Curd firmness 30 min after enzyme addition (a30, mm) which corresponds to the width of the graph 30 min after rennet addition;
- Curd firmness 60 min after enzyme addition (a60, mm) which corresponds to the width of the graph 60 min after rennet addition. (Stocco, et al., 2018)

These parameters describe the milk coagulation process: the enzymatic or primary phase after the chymosin effect, the non-enzymatic or secondary phase after the casein aggregation, and the last phase after the gel structure formation. The MCP is the determining factor in cheese quantity and quality and is highly variable (De Marchi, et al., 2007).

2 AIM OF THE THESIS

This study aimed to characterize the 'La Dinara' farm of dairy goats from a genetic and phenotypic point of view. In terms of genetic research, an evaluation of mating management from pedigree data was attempted.

From a phenotypic point of view, the milk was analysed and compared to samples from the BIO4VERBA project to evaluate the difference between ‘La Dinara’ farm located in Terenzo (PR) and farms located in Lombardy region considering two different housing conditions (indoor and grazing).

3 MATERIALS AND METHODS

3.1 EXPERIMENTAL DESIGN

For the genetic studies of this thesis, the pedigree data of the 'La Dinara' farm of Terenzo (PR) was analysed. A dataset including 224 animals was initially created and analysed using the Endog v4.8 software.

Regarding the farm's phenotypic analyses, in this study, a total of 28 milk samples were collected, during the evening milking. Two sampling sessions were carried in May and July, covering two different housing conditions: indoor conditions for the first sampling and grazing in the second one. Fourteen animals were sampled at each sampling session.

For the statistical analyses, the results obtained from the analysis of 38 samples from the DBA.AD002.565 BIO4VERBA project analysed with the same methodologies were added to evaluate potential differences between the milk produced by 'La Dinara' farm and other farms. The Camosciata delle Alpi goat breed from the Lombardy region was one of four breeds examined by the BIO4VERBA project in two samplings: one during the housing period and the other during grazing and therefore used for comparison.

3.2 GENETIC VARIABILITY

3.2.1 Data available and Reference Population

All the data were provided by the dairy goat farm 'La Dinara'. The information was acquired from a pedigree that the farm's owner has been keeping since 2016.

The pedigree database contained 225 animals (TP: total population) of which 28 currently alive (RP: reference population): 26 females (92.85%) and 2 males (7.15%).

Reference population (RP) is defined as the total number of goats currently alive, whose pedigrees were traced back to the earliest recorded ancestors. In addition to the RP, the current breeding population (BP) was built considering alive male and females used for reproduction purpose. To build the BP two filters were applied: males and females alive with at least one offspring (Table 1) (Ablondi, et al., 2020).

The completeness of pedigree information was investigated using the equivalent complete generation (CGE), which is computed as the sum of $(1/2)^n$, where n is the number of generations between individuals and each known ancestor (Maignel, et al., 1996).

Table 1- Description of the data available in the entire pedigree dataset (TP), in the reference population (RP) and in the breeding population (BP).

Parameters	**TP[1]**	**RP[2]**	**BP[3]**
Numbers of goats	224	28	41
Numbers of males	104	2	6
Numbers of females	120	26	35
Numbers of goats with no progeny	184	11	0

[1]TP = total population, total number of animals present in the database; [2]RP = reference population, number of pigs currently alive; [3]BP = breeding population, breeding animals in the reference population.

3.2.2 Inbreeding and Average relatedness

At first, an excel dataset was made with all the information obtained from the pedigree book. Using the ENDOG v4.8 software, inbreeding and average relatedness were estimated.

The inbreeding coefficient (F) is the probability of an animal to be homozygous for a locus by descendant and was computed according to Meuwissen, et al., 1992. The rate of the increase in inbreeding (ΔF) was also calculated per generation, as well as the F.

The formula used to calculate the rate of the increase in inbreeding is:

$$\Delta F = \frac{F_t - F_{t-1}}{1 - F_{t-1}}$$

With a generation interval equal to 3.2 years.

The average relatedness coefficient (AR) of each individual is defined as the probability that an allele randomly chosen from the whole population in the pedigree belongs to a given animal.

According to J.P Gutiérrez & F. Goyache (Gutierrez, et al., 2005) ENDOG v4.8 uses an algorithm to compute the individual average relatedness (AR) coefficients.

Let a vector **c'** defined as:

$$\mathbf{c'} = (1/n)\ \mathbf{1'A} \qquad \textbf{[1]}$$

A being the numerator relationship matrix of size n x n. On the other hand, the numerator relationship matrix can be obtained from the **P** matrix, where p_{ij} is equal to 1 if *j* is parent of *i*, and 0 otherwise, which sets the parents of the animals (Quaas, 1976), by:

$$\mathbf{A} = (\mathbf{I} - ½\ \mathbf{P})^{-1}\ \mathbf{D}\ (\mathbf{I} - ½\ \mathbf{P'})^{-1} \qquad \textbf{[2]}$$

where **D** is a diagonal matrix with non-zero elements obtained by:

$$d_{ii} = 1 - ¼\ a_{jj} - ¼\ a_{kk} \qquad \textbf{[3]}$$

j and k being the parents of the individual i.

From **[2]**, $\mathbf{A}\ (\mathbf{I} - ½\ \mathbf{P'}) = (\mathbf{I} - ½\ \mathbf{P})^{-1}\ \mathbf{D}$

Premultiplying by (1/n) **1'**:

$$(1/n)\ \mathbf{1'}\ \mathbf{A}\ (\ \mathbf{I} - ½\ \mathbf{P'}\ ) = (1/n)\ \mathbf{1'}\ (\ \mathbf{I} - ½\ \mathbf{P}\ )^{-1}\ \mathbf{D}$$

and using **[1]**:

$$\mathbf{c'}\ (\mathbf{I} - ½\ \mathbf{P'}) = (1/n)\ \mathbf{1'}\ (\mathbf{I} - ½\ \mathbf{P})^{-1}\ \mathbf{D}$$

Multiplying **c'** into the parenthesis and isolating **c'**:

$$\mathbf{c'} = (1/n)\ \mathbf{1'}\ (\mathbf{I} - ½\ \mathbf{P}\ )\text{-}1\ \mathbf{D} + ½\ \mathbf{c'}\ \mathbf{P'}\ \mathbf{[4]}$$

3.3 MILK ANALYSIS

3.3.1 Data collection

A total of 14 Camosciata breed goats reared in 'La Dinara' farm were sampled twice. In order to highlight any changes in milk quality, two samples were taken two months apart. The first sampling was made on May 15, 2023 and the second sampling took place on July 13, 2023. The animals were kept in indoor condition for the first sampling, while during the second sampling they were let out into the pasture during the day. The first sampling involved the analysis of 14 individual samples, whereas the second sample involved the analysis of 13 individual samples and a sample of mass milk. All the samplings involved the sampling of 100ml of milk per each animal during the evening milking, with the same sampling methodology.

3.3.2 Milk quality traits

The chemical composition of milk and serum are expressed in percentages of protein, fat, casein, and lactose. The chemical composition was analysed using Milkoscan FT3 (FOSS, Italy).

Total (SCC) ad differential (DSCC) somatic cells was determinate by a Fossomatic™ 7DC (FOSS, Italy). The total bacterial count (IBC) and colony forming units (UFC) and was determinate by the use of a BactoScan (FOSS, Italy).

3.3.3 Milk yield traits

The 9-MilCa proposed by Cipolat-Gotet et al. (Cipolat - Gotet , et al., 2016) was used to measure single-point milk coagulation properties (MCP), nutrient recovery traits (%REC) , and predicted cheese yield traits (%CY). The following procedure was performed with two replicates per each individual milk sample.

Each milk replicate (9 mL) was poured into a glass tube, inserted into the modified sample rack of the lactodynamograph instrument, heated to 35°C for 15 min, and mixed with 0.2 mL of a rennet solution (*Figure 4*). The sample rack was then moved to the lactodynamograph set at 35°C, for a 30-min duration test. At the end of the analysis, coagulated replicates of milk samples were manually cut using a steel spatula and moved to the heater at 55°C, for the 30-min curd-cooking phase. During the cooking phase, each replicate was subjected to a further manual cutting by the same operator, and the curd particles were allowed to sit quiescently in their own whey after cutting. At the end, each glass tube was removed from the sample rack, and the curd was separated from the whey. The curd was pressed and suspended above the whey for 15 min at room temperature to allow the draining. The obtained curd and whey were weighed using a precision scale. Whey composition was assessed using an infrared spectrophotometer (MilkoScan FT3, Foss Electric). The weights of the curd and whey (g) and the chemical composition of milk and whey also allowed us to estimate curd composition by calculating the difference between milk and whey contents. The cheese yield (%CY) traits were $\%CY_{CURD}$, $\%CY_{SOLIDS}$, and $\%CY_{WATER}$, calculated as the ratios of the weight (g) of fresh curd, curd DM, and water retained in curd, respectively, to the weight of the milk processed (g), multiplied by 100. The component recovery (%REC) traits were as follows: $\%REC_{PROTEIN}$, $\%REC_{FAT}$, and

%REC_{SOLIDS}, calculated as the ratios of the weight (g) of the curd components (protein, fat, and DM, respectively) to the same component of milk (g), and multiplied by 100. (Vacca, et al., 2018)

The classical formula for cheese yield (%CYCURD, %) can be written as follows:

$$\%CY_{CURD} = \frac{curd\ (g)}{milk\ (g)}\ x\ 100$$

Cheese yield was also calculated for total solids (TS; %CYSOLIDS, %) and water (%CYWATER, %) of the curd, as follows:

$$\%CY_{SOLIDS} = \frac{milk\ TS\ (g) - whey\ TS\ (g)}{milk\ (g)}\ x\ 100$$

$$\%CY_{WATER} = \frac{milk\ water\ (g) - whey\ water\ (g)}{milk\ (g)}\ x\ 100$$

Considering the weight (g) of the individual components of the milk and curd, the recoveries (REC; %) of milk protein, fat, and TS in the curd were calculated as:

$$\%REC_{PROTEIN} = \frac{milk\ protein\ (g) - whey\ protein\ (g)}{milk\ protein\ (g)}\ x\ 100$$

$$\%REC_{FAT} = \frac{milk\ fat\ (g) - whey\ fat\ (g)}{milk\ fat\ (g)}\ x\ 100$$

$$\%REC_{SOLIDS} = \frac{milk\ TS\ (g) - whey\ TS\ (g)}{milk\ TS\ (g)}\ x\ 100$$

Figure 4-Addition of rennet solution in MilCa laboratory in University of Parma.

3.3.4 Milk coagulations properties

A Lactodynampgraph LDG V2.0 (Ma.Pe System, Italy) was used to assess MCP. The procedure was performed with 2 replicates each individual milk sample.

Rennet was diluted in distilled water to obtain a solution of 1.2% (wt/vol). The instrument records four single-point recorded MCP: Rennet coagulation time (RCT, min), the time to a curd firm ness (CF) of 20 mm (k20, min, curd firmness 30 min after enzyme addition (a30, mm) and curd firmness 60 min after enzyme addition (a60, mm)(Stocco, et al., 2018).

3.4 STATISTICAL ANALYSIS

At the beginning of the statistical analysis all the data obtained were passed through a quality control. Replicates (two replicates per goat) of the data from the cheesemaking procedure were averaged; the SCC was log-transformed to SCS, using the following formula, $[Log_2(SCC \times 10^{-5}) + 3]$ to achieve normality and homogeneity of variances according to Ali, et al., 1980 and all outliers were eliminated (Ali, et al., 1980).

Also IBC was log-transformed to LBC (logarithmic bacterial count) using the formula $\log_{10}$ (total bacterial count/1,000) described by Vacca, et al., 2018.

R Statistical Software was used to analyse all experimental data from milk analyses performed in the lab. This software used many packages, each with a different set of features:

- *readxl*: Used for reading Excel files;
- *dplyr*: Used for data manipulation;
- *tidyverse*: A collection of packages for data manipulation and visualization;
- *skimr*: Used for generating summary statistics and data descriptions.

Three distinct kinds of analyses were performed:

- Shapiro Wilk test: to test the normality of data (no package used, basic R functions);
- ANOVA: analysis of variance with evaluation of the effect of the factors region, farm, sampling (and interactions between them) on our data (packages used *emmeans*: Used for conducting post hoc tests in ANOVA and *ggplot2*: Used for creating data visualizations). Although it is recognized that parity and days in milk could impact the results, no data were available for the BIO4VERBA observations and thus we could not include those correcting factors in the model.

4 RESULTS AND DISCUSSION

4.1 GENETIC VARIABILITY

The average CGE appears to be 1.415 for TP and 0.731 for BP, these results are rather low. Given the lack of knowledge of the founding subjects' history, a low CGE was expected.

Figure 5 defines the population growth since the farm's foundation by sex and dividing animals in breeding and non-breeding ones. The graph in *Figure 5* indicates a population increase at first, followed by a stabilisation of the total number of animals per year.

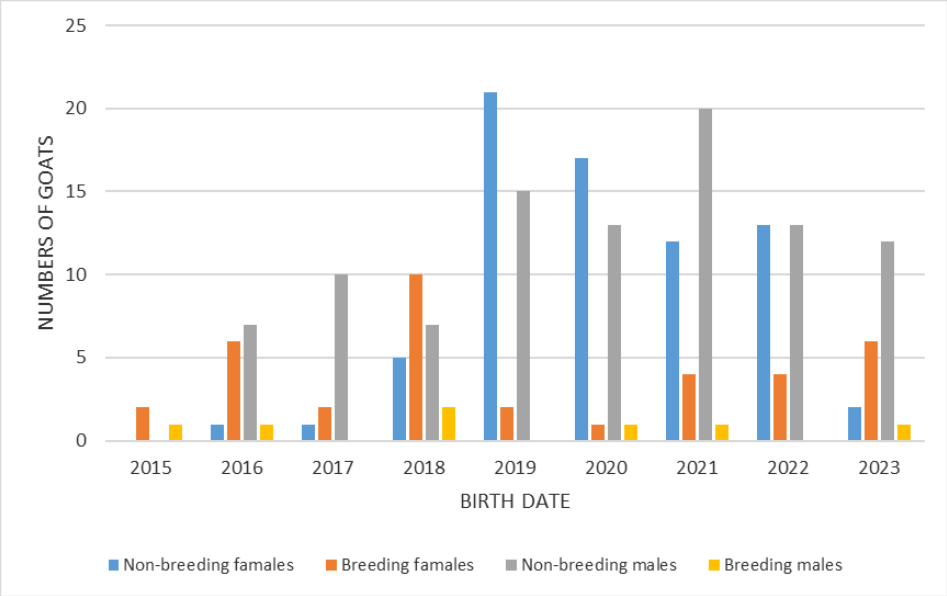

Figure 5 - Population trend from the foundation of the farm to today

The results of the genetic variability study demonstrated an excellent management of mating in the farm and a meticulous selection of individuals to serve as breeders. In fact, it has been demonstrated that the inbreeding of breeding subjects is zero in all the generations. On the other hand, *Figure 6* shows that breeding subjects' average relatedness is rising over time which is expected based on the small number of funder animals; in fact,

especially on small farms, it is possible to restrict but not completely eliminate the growth in the average relatedness between animals in a population that is susceptible to selection.

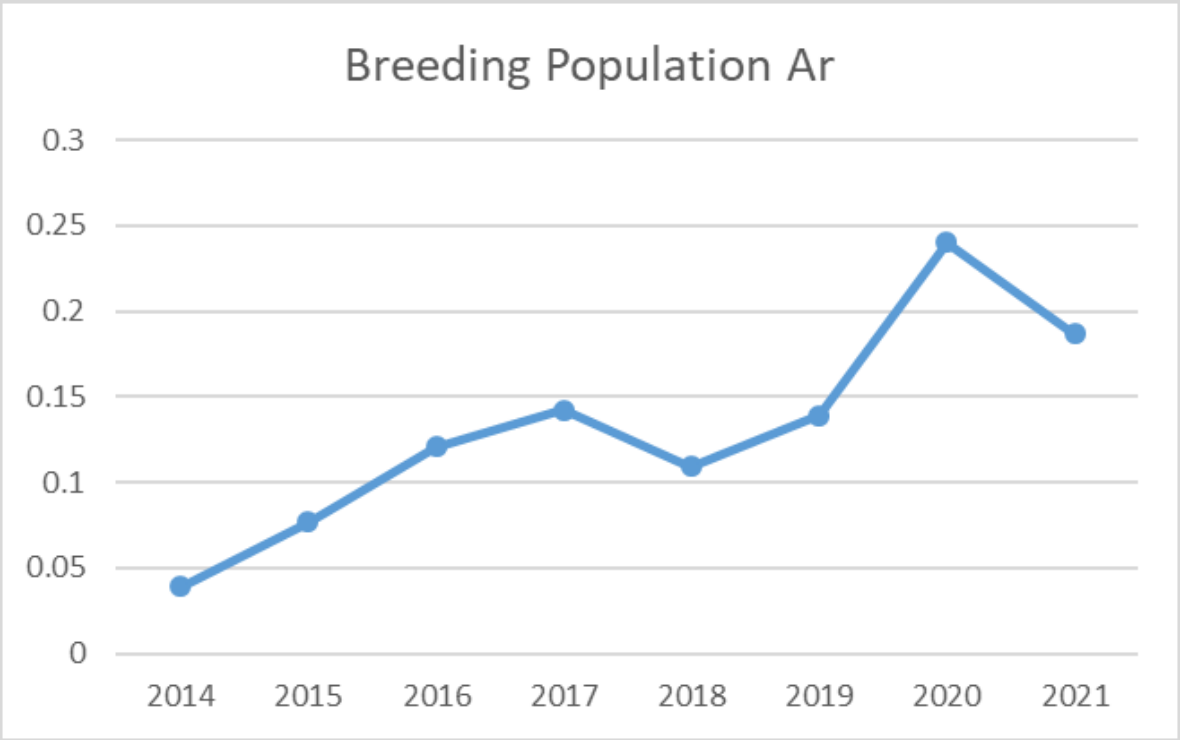

Figure 6 - Average relation trend in BP.

The rate of inbreeding per generation (Δ*F*) between the last and the penultimate generation, assuming a mean GI of 3.2, was equal to 1.00 % in the TP. The Food and Agriculture Organization (FAO) stated that the value of ΔF should not exceed 1% to avoid substantial loss of genetic material over time (Sherf, 2000).

Since the company's first subjects were from separate farms, the initial inbreeding is 0; however, it is noticeable that the amount of inbreeding per generation is rising in time.

4.2 MILK ANALYSIS

In terms of milk studies, it was decided to conduct a comparison between two distinct geographical areas using the data from the BIO4VERBA project.

In attempt to show the potential interaction between farm and farming housing system (grazing and indoor housing), the housing condition factor and the territorial factor were related in the setting of the analysis (here after called as “Effect of Housing Condition and Region”).

The farms were split up into two groups in order to conduct evaluations. Within the first group is the breeding farm 'La Dinara', No. 1, which is situated in the Emilia Romagna; the other group consists of the three farms in Lombardy that were the subject of the BIO4VERBA project's examination (these herds were defined as No. 2, 3 and 4).

4.2.1 Descriptive statistic

Table 2 summarizes descriptive statistics of milk composition, %REC, %CY and milk coagulation properties of the 65 goat milk samples analysed. Comparing our data with those obtained from the study of Vacca , et al., 2018, which examined the milk of six distinct goat breeds, we find that the values obtained from our study are in the range of what obtained in the study mentioned above. A complete list of the descriptive statistics found in Vacca et al., 2018 can be found in *Table 3*.

Table 2 - Descriptive statistics of milk composition, somatic and differential cells, nutrient recovery, cheese yield and milk coagulation properties of farm No.1, 2, 3, and 4.

Traits	N	Mean	CV	Min	Max	Median
Milk composition						
Fat, %	64	3.50	26	1.74	6.07	3.38
Protein, %	64	2.90	10	2.33	3.57	2.86
Casein, %	64	2.27	13	1.78	2.92	2.24
Casein index[1]	64	77.90	3	72.10	82.20	77.80
Lactose, %	64	4.32	5	3.89	4.78	4.30
Total solids, %	64	11.10	10	9.19	14.20	11.00
UFC [2], U	64	58.20	210	1.00	659.00	11.00
LBC[3]	64	-1.17	-71	-2.70	0.55	-1.18
SCS[4]	64	5.58	39	0.94	9.93	5.09
DSCC [5], %	64	73.30	24	28.00	94.10	80.30
Cheese yield (%CY), %						
$\%CY_{CURD}$	65	12.80	16	8.14	17.00	12.40
$\%CY_{SOLIDS}$	65	5.55	18	3.87	8.22	5.38
$\%CY_{WATER}$	65	7.23	21	3.73	11.00	6.86
Nutrient recovery (%REC), %						
$\%REC_{FAT}$	65	81.80	6	69.20	91.20	81.40
$\%REC_{PROTEIN}$	65	78.70	2	74.60	82.30	78.80
$\%REC_{SOLIDS}$	65	49.40	8	40.70	58.60	49.00
Milk coagulation properties (MCP)						
RCT, min	65	14.00	41	6.94	34.80	12.40
K_{20}, min	65	4.55	59	1.12	16.70	3.88
A_{30}, mm	65	33.90	32	4.85	54.40	36.30
A_{60}, mm	65	33.40	32	5.39	54.40	35.20
A_{MAX}, mm	65	52.40	14	22.10	58.20	42.10

[1] *Casein index: casein to protein ratio.*
[2] *UFC = Colony Forming Units.*
[3] *LBC = logarithmic total bacterial count = log_{10}(total bacterial count/1,000).*
[4] *SCS = log2 (SCC × 10−5) + 3.*
[5] *DSCC =Differential somatic cells count.*

Nevertheless, as average value we observed that the Fat, Protein, Casein, Casein Index and lactose are lower in this study compared to goat milk analysed by Vacca et al., 2018 (*Table 3*). In the case of LBC and SCS the farms analysed in this study demonstrated lower values and therefore better herd management from a health point of view compared to Vacca et al., 2018.

Regarding the CY traits we can observe that all the data from this study are slightly lower than those obtained by Vacca et al.,2018. However, for the REC traits a slightly higher recovery of REC_{FAT} (81.8% vs 80.5%) was observed but lower $REC_{PROTEIN}$ (78.7% vs 81.5%) and REC_{SOLIDS} (49.4% vs 55.7%) (*Table 3*).

Table 3 – Descriptive statistic of milk composition, hygienic quality and milk yield traits of the study by Vacca et al., 2018.

Traits	Goat Milk Vacca et al., 2018	
	Mean	CV
Milk composition		
Fat, %	4.59	35
Protein, %	3.59	15
Casein, %	2.82	18
Casein index [1]	0.78	4
Lactose, %	4.66	6
Total solids, %	13.74	14
LBC [2]	1.8	35
SCS [3]	5.61	46
Cheese yield (%CY), %		
$\%CY_{CURD}$	15.7	20
$\%CY_{SOLIDS}$	7.7	23
$\%CY_{WATER}$	8.00	20
Nutrient recovery (%REC), %		
$\%REC_{FAT}$	80.5	8
$\%REC_{PROTEIN}$	81.5	3
$\%REC_{SOLIDS}$	55.7	10

[1]Casein index: casein to protein ratio.
[2]LBC = logarithmic total bacterial count = $\log_{10}$ (total bacterial count/1,000).
[3] SCS = log2 (SCC × 10^{-5}) + 3.

4.2.2 Effect of region and housing condition on milk traits

Table 4 presents the analysis of variance using the ANOVA method for fixed effects of milk and cheese-making traits. The effects of housing conditions were assessed - first sampling in indoor housing and second sampling on grazing - and the farms were divided into two groups. The first group, identified as Reg1, represents the 'La Dinara' farm, while the second group comprises three farms located in the Lombardy region. And this grouping has been defined as the region effect.

Table 4- Analysis of variance (F-values and significance) for fixed effects of milk and cheese-making traits.

Traits	Region	Housing condition	Farm	Region:Housing condition
Milk composition				
Fat, %	1.9	0.1	4.7*	3.7
Protein, %	0.8	4.7*	0.1	0.3
Casein, %	3.2	3.3	0.1	0.5
Casein index [1]	26.9***	0.1	0.2	1.5
Lactose, %	0.3	8.1***	4.0*	2.1
Total solids, %	4.9*	2.6	1.9	1.3
UFC [2], U	7.5***	0.5	0.4	0.8
LBC [3]	94.4***	5.1*	10.2***	0.3
SCS [4]	9.2**	6.6*	3.209*	0.1
DSCC [5], %	9.6***	2.2	0.8	1.0
Cheese yield (%CY), %				
$\%CY_{CURD}$	2.9	27.7***	2.0	0.4
$\%CY_{SOLIDS}$	2.1	4.1*	2.1	1.9
$\%CY_{WATER}$	14.0***	38.4***	0.9	4.1*
Nutrient recovery (%REC), %				
$\%REC_{FAT}$	0.0	5.9*	0.8	0.0
$\%REC_{PROTEIN}$	0.2	7.5**	0.8	0.8
$\%REC_{SOLIDS}$	0.1	5.2*	1.9	2.6
Milk coagulation properties (MCP)				
RCT, min	7.6**	0.6	3.1	3.3
K_{20}, min	9.2**	6.3*	1.3	3.4
A_{30}, mm	10.3**	6.9*	2.1	6.5*
A_{60}, mm	14.5***	15.4***	0.2	22.3***
A_{MAX}, mm	4.7*	12.7***	1.1	2.2

[1] Casein index: casein to protein ratio.
[2] UFC = Colony Forming Units.
[3] LBC = logarithmic total bacterial count = log_{10}(total bacterial count/1,000).
[4] SCS = log2 (SCC × 10−5) + 3.
[5] DSCC =Differential somatic cells count.
**P < 0.05; **P < 0.01; ***P < 0.001.*

From the analysis of variance, it was found that among the factors, region (which in our case was used to distinguish 'La Dinara' farm from the remaining ones located in Lombardy) was significant on Casein index, TS, UFC, LBC, SCS and DSCC and on CY_{WATER}, RCT, K_{20}, A_{30}, A_{60} and A_{MAX}.

The housing condition significantly influenced %CY, %REC, all MCP traits except RCT and protein, lactose, LBC and SCS.

The farm factor influenced only some traits such as fat, lactose, LBC and SCS.

The interaction between housing condition and region significantly influenced CY_{WATER}, A_{30} and A_{60}.

4.2.3 Milk composition

From a milk composition point of view, it can be observed that the farm significantly influences the fat and lactose traits, as previously reported in *Table 4*.

In *Figure 7* are reported the Boxplots of Fat and Lactose according to the farm effect.

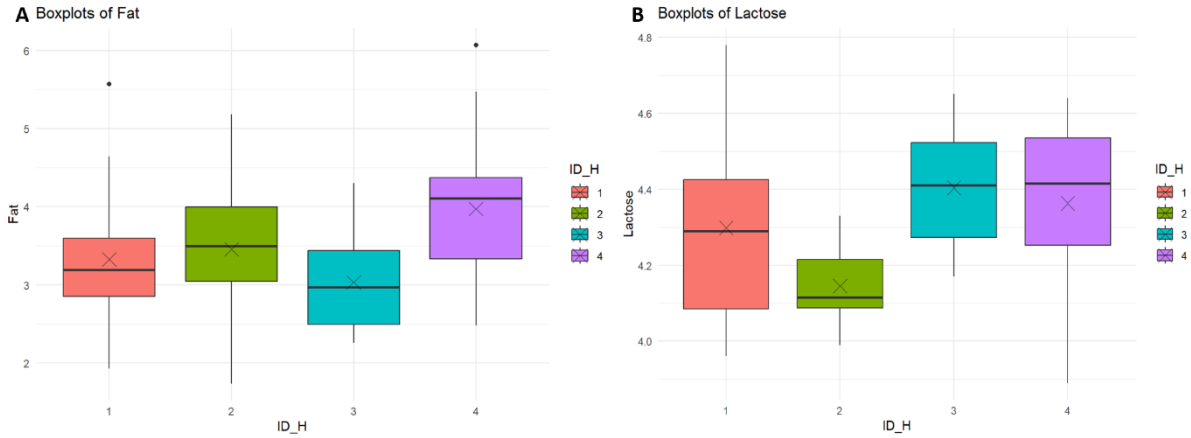

Figure 7 - Effect of farm on Fat (A) and Lactose (B).

From these two graphs we can highlight that the percentage of fat (*Figure 7*A) is slightly different between the first three farms while it is much higher and more varied in farm number 4 which is one of the farms located in Lombardy. In terms of lactose, farms 1, 2,

and 3 demonstrate good lactose percentage with significant variability ranging from 4.1 to 4.5, but farm 2 presents lower lactose with less variability. These differences can be justified by the differences in management and feeding of the four farms.

Rather, the housing condition affects lactose and protein as demonstrated by the boxplots in *Figures 8* and *9.*

In the boxplot in *Figure 8*, the protein values significantly differed comparing the the sampling in indoor housing and in the pasture. The boxplot highlights that there is a lower percentage of protein in pasture with an average of 2.7% and an average of 2.9% in housing condition.

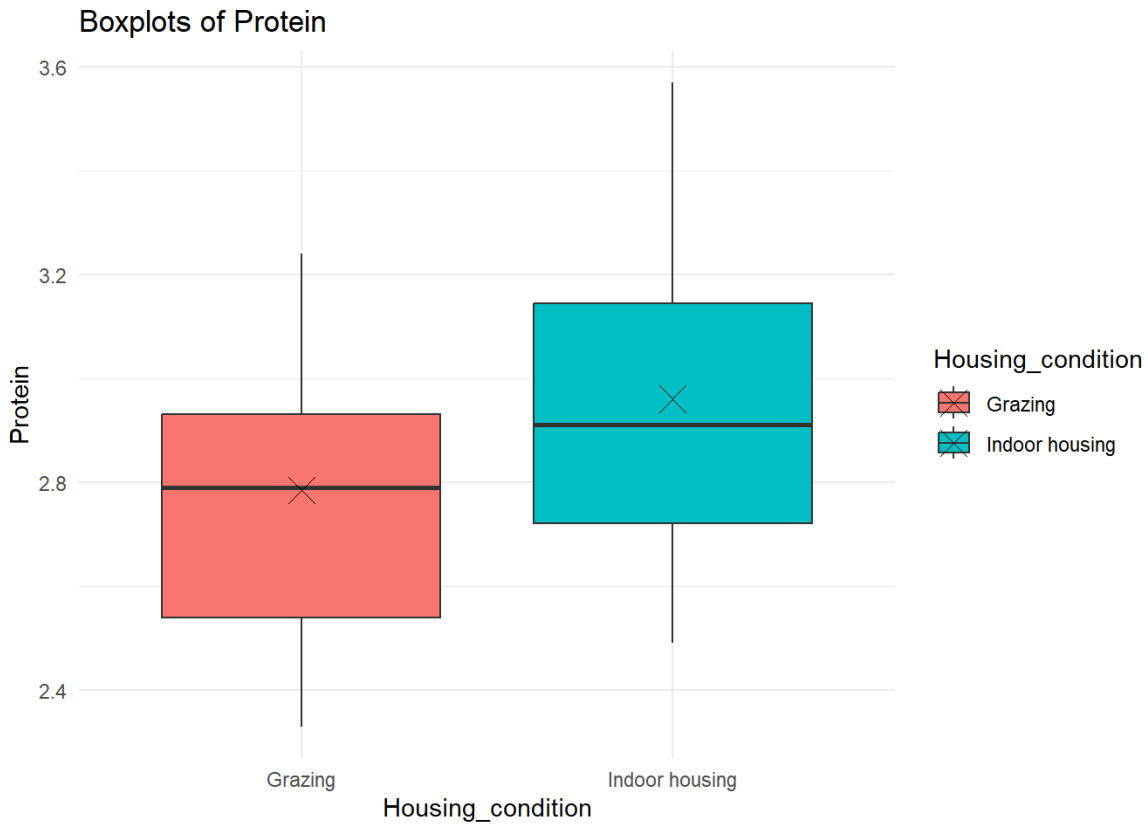

Figure 8 – Effect of housing condition on Protein.

Figure 9 shows the percentage of lactose comparing the two housing conditions, also in this case it can be highlighted that there is a lower percentage of lactose when animals are on pasture compared to animals in indoor housing.

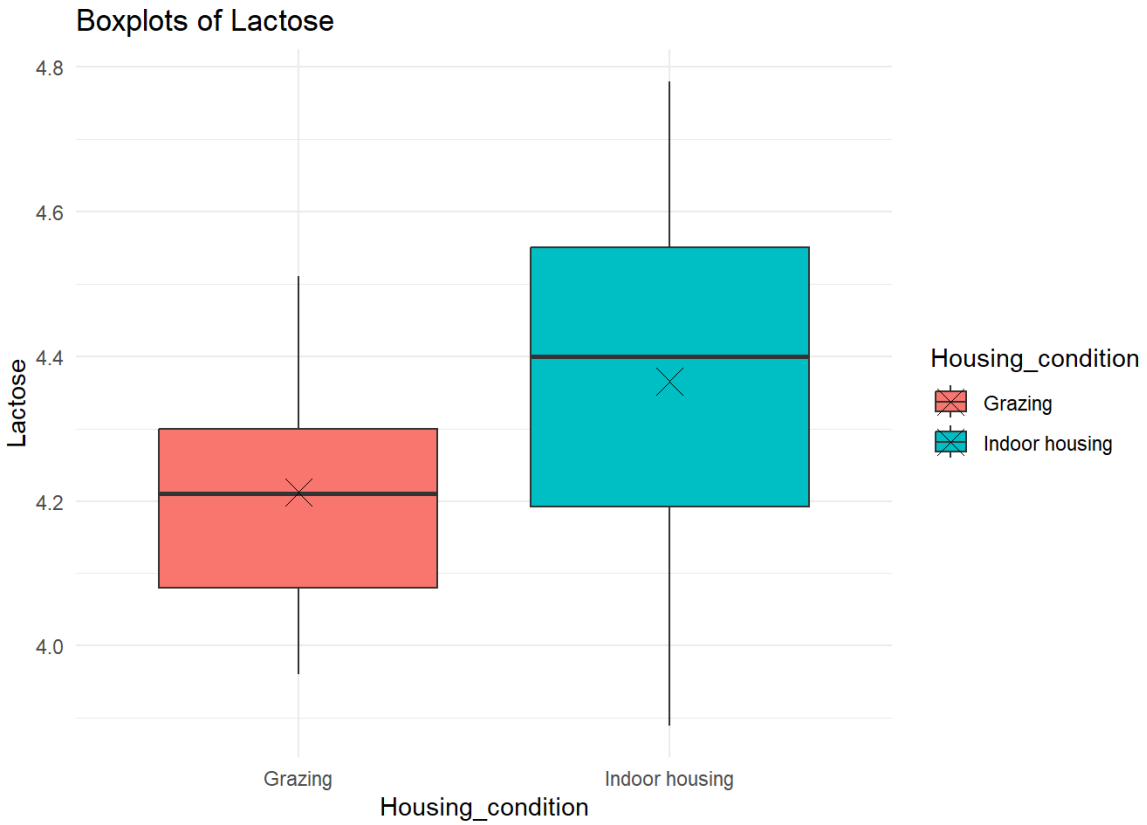

Figure 9 - Effect of housing condition on Fat.

A lower percentage of lactose and protein in pasture-fed animals is also demonstrated by Oprean et al., 2011 where the changes in the composition of goat's milk were compared in different types of feeding. In Oprean et al., 2011 it is stated that when the goats were kept on pasture without concentrated supplementation, lactose content was the lowest value and that the protein content of milk was significantly lower in this group compared to other conditions (Oprean, et al., 2011).

By analyzing the hygienic traits UFC,LBC , SCS, DSCC we can observe that they are significantly influenced by the region effect and the housing condition effect.

For the region factor, as we can see in *Figure 10*, there is very good management of animal health in Lombardy region (Reg.2) while we find slightly higher values in La Dinara farm (Reg.1).

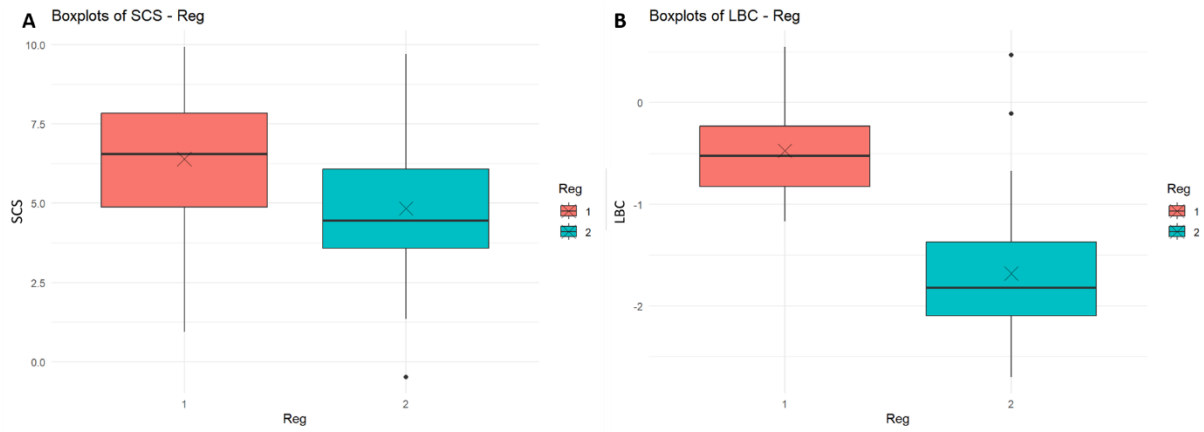

Figure 10 - Effect of Region on SCS (A) and LBC (B).

The impact of the housing condition factor on the SCS trait is graphically shown in *Figure 11*. We can observe that the somatic cell score has significantly increased; sampling in grazing results in a score of 6.5 whereas indoor housing results in a score of 4.9, which correspond respectively to about 1'200'000 cells/ml and 400'000 cells/ml.

Figure 11- Effect of Housing Condition on SCS.

Regarding the effect of housing conditions on logarithmic bacterial count (LBC), *Figure 12* highlights an increase similar to that found for SCS. Indeed, we find an LBC of -1.4 for indoor habitation and -0.7 for grazing.

Figure 12 - Effect of Housing Condition on LBC.

Given this, we can conclude that, when compared to the result reported by Vacca et al. (2018), which show an average value of SCS of 5.61 and an LBC of 1.80, our data demonstrate excellent herd management from a health perspective which is evident in both housing conditions, irrespective of the specific farm under consideration. However, a clear worsening of hygiene traits during grazing can be noted for both SCS and LBC. This result is attributable to the fact that during pasture the goat's udder is more likely to develop lesions and come into contact with bacteriological agents.

The study by Pazzola et al., 2022 produced similar, but less significant results, showing an LBC of 2.24 for extensive farming systems and 2.09 for intensive farming systems, as well as a SCS of 6.67 for extensive and 6.57 for intense.

4.2.4 Milk yield traits

Cheese yield curd (CY_{CURD}), which is separated into CY_{WATER} and CY_{SOLID}, and recovery (REC%), or the quantity of milk components recovered in the curd (REC_{FAT}, $REC_{PROTEIN}$ and REC_{SOLID}), are considered the cheesemaking traits.

It is recognized that %CYCURD depends primarily on the TS content of the milk, its recovery in curd, and the retention of water in cheese (Cecchinato, et al., 2016).

Regarding the CYCURD we can observe that there is a significant variability given by the housing condition factor (*Figure 13*) this is also reflected in the CY_{WATER} and CY_{SOLID} as they are the two factors that compose it.

Boxplot represented in *Figure 13* highlights that there is a clear decrease in the percentage of CY_{CURD} in the animals that were sampled during the grazing period, with an average of 11.3%, while there is a greater percentage of cheese yield in curd for the animals in indoor housing with an average of 13.5%.

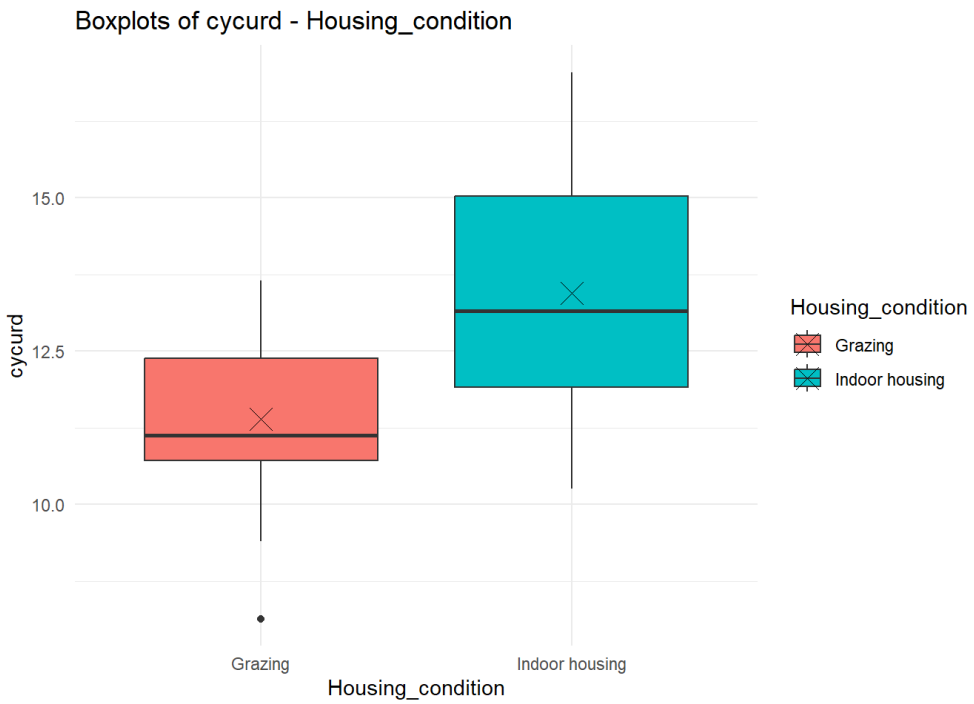

Figure 13- Effect of Housing Condition on CYCURD.

The same difference can be observed in CY_{WATER}, with an average of 6.2% in pasture and 7.7% in indoor conditions, and in CY_{SOLIDS}, with an average of 5.1% in pasture and 5.7% in indoor conditions.

Figure 14 shows the results of the CY_{WATER} traits in the 'La Dinara' farm and in the farms located in the Lombardy region under grazing or indoor conditions. A significant variability of CY_{WATER} for the factor of interaction between the region and the housing condition is shown.

Two sections are shown in the boxplot in *Figure 14*: the first section deals with the La Dinara farm located in Emilia Romagna, identified as Reg1, and the second one shows the variability of the others farms located in Lombardy, identified as Reg2 comparing the two housing condition.

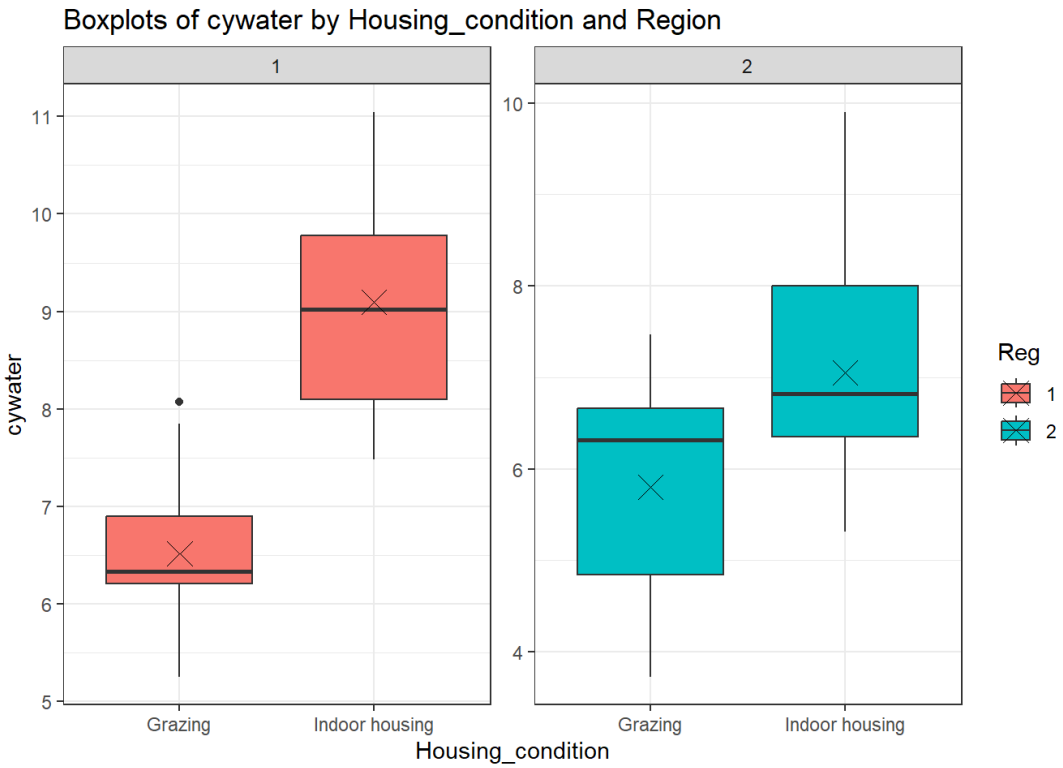

Figure 14 – Effect of Housing Condition and Region in %CY_{WATER}.

Therefore, it can be concluded that Emilia Romagna's breeding exhibits a larger difference between the CY_{WATER} in pasture, with an average of 6.5%, and the indoor condition, with an average of 9.1% compared to the other farms located in Lombardy.

The farms in Lombardy still show differences but significantly less than those found in 'La Dinara' with an average CY_{WATER} of 5.9% for pasture and 7% for indoor conditions.

However, the same variability of CYCURD for housing conditions was not found in Pazzola, et al., 2022 where it is stated that the Farming System did not have a significant variation on these traits as we can see in *Figure 15*.

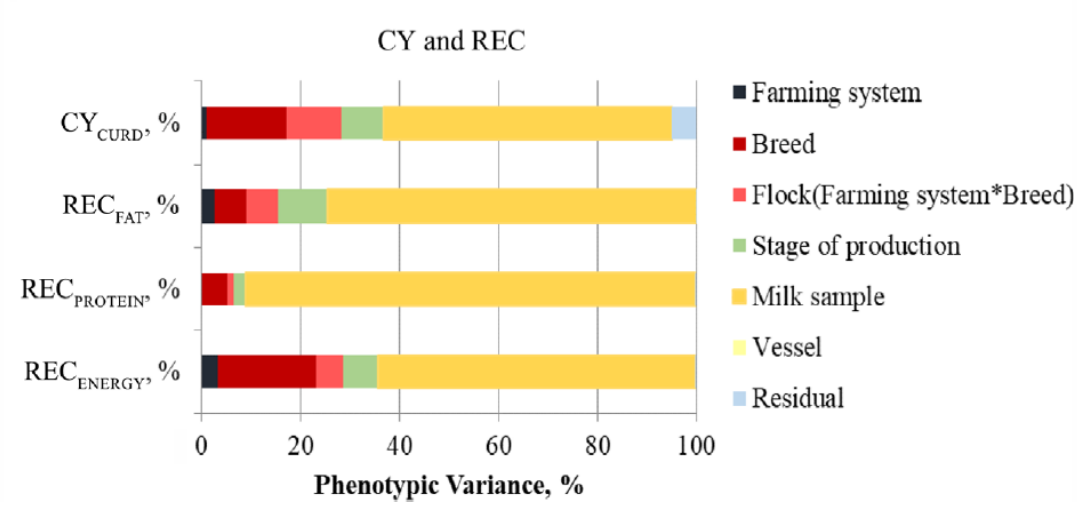

Figure 15 - Proportion of the phenotypic variance of cheese yield and recovery traits (CY% and REC%) of goat bulk milk. (Pazzola, et al., 2022)

As with %CY, significant variation was found for recovery traits, such as $\%REC_{FAT}$, $\%REC_{PROTEIN}$ and $\%REC_{SOLIDS}$, as a result of the impact of housing conditions.

This variation is more significant for the $\%REC_{PROTEIN}$ trait as we can see in *Figure 16* where an average of $\%REC_{PROTEIN}$ of 77.9% for pasture and 79.1% for indoor housing was found.

As highlighted in *Figure 15* Pazzola, et al., 2022 did not find the same significant variability for $\%REC_{PROTEIN}$. We can instead note that in Pazzola et al., 2022 the farming system had a small effect on the variation in REC_{FAT}.

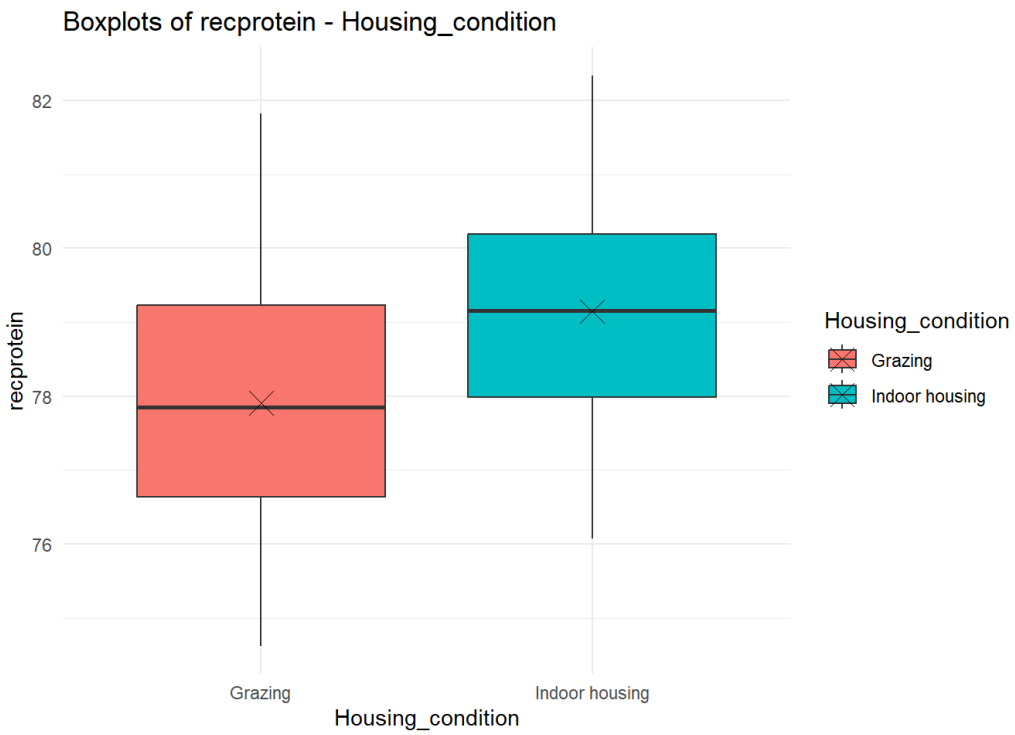

Figure 16 - Effect of Housing Condition on %REC$_{PROTEIN}$.

Similarly to Pazzola et. al, 2022, in this study, the effect of the housing condition proved to be significant on the variability of the %REC$_{FAT}$ as we can observe from the boxplot depicted in *Figure 16*.

It is observed that %REC$_{FAT}$ during grazing has an average of 79.6% and in indoor conditions of 82.8%.

Pazzola, et al., 2022 affirm that regarding milk coagulation and cheesemaking traits, the milk samples from extensive farms exhibited a slower progression of curd firming, a lower potential curd firmness, and lower percentages of fat recoveries in the fresh curd.

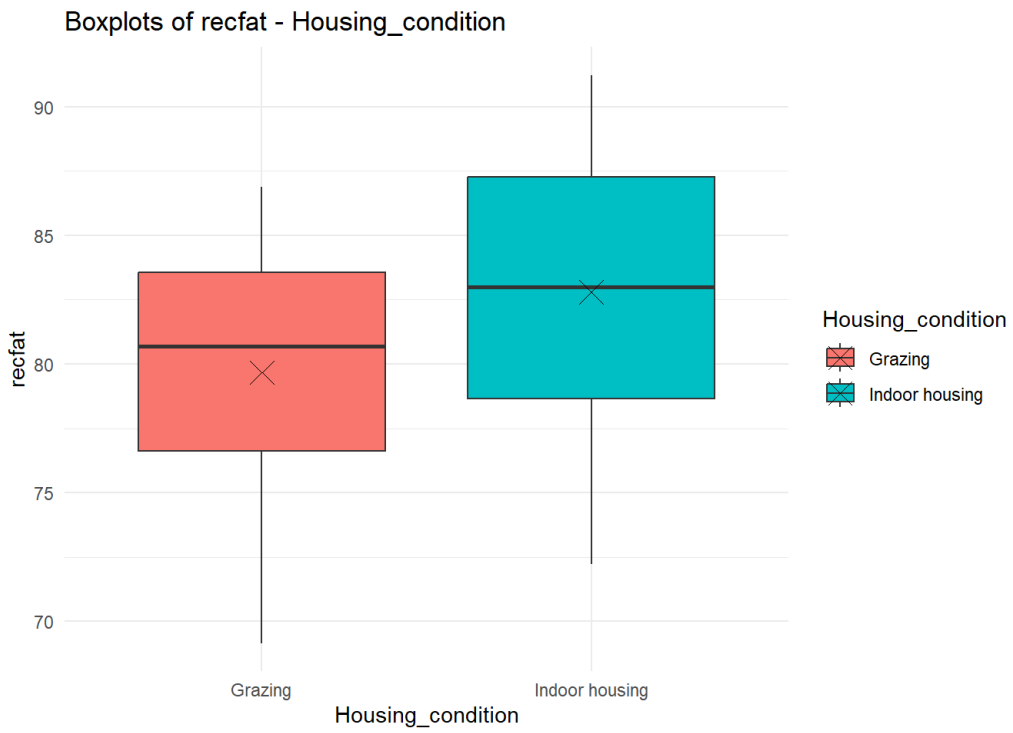

Figure 17- Effect of Housing Condition on %REC_{FAT}.

4.2.5 Milk coagulation properties

Regarding milk coagulation properties, we highlighted that the interaction between the region and the housing condition was significant for the two parameters A30 (firmness of the curd after 30 minutes) and A60 (firmness of the curd after 60 minutes).

By examining *Figure 18*, we can observe that between the Emilia Romagna region and the Lombardy region we find two distinct statistical trends. In 'La Dinara' farm identified as Reg1, there is a significant difference in average A30 values between sampling conducted during grazing (average values 22.4mm) and indoor condition (average values 36.2mm). Thus, it can be observed that in the indoor situation, the curd's firmness after 30 minutes is significantly larger.

In the farms located in Lombardy identify as Reg2, there is no significant variability in A30 values and curd firmness in indoor situations is slightly lower than during grazing. In fact,

the average values of A30 appear to be 37.6mm in the pasture and 36.9mm in indoor conditions (*Figure 18*).

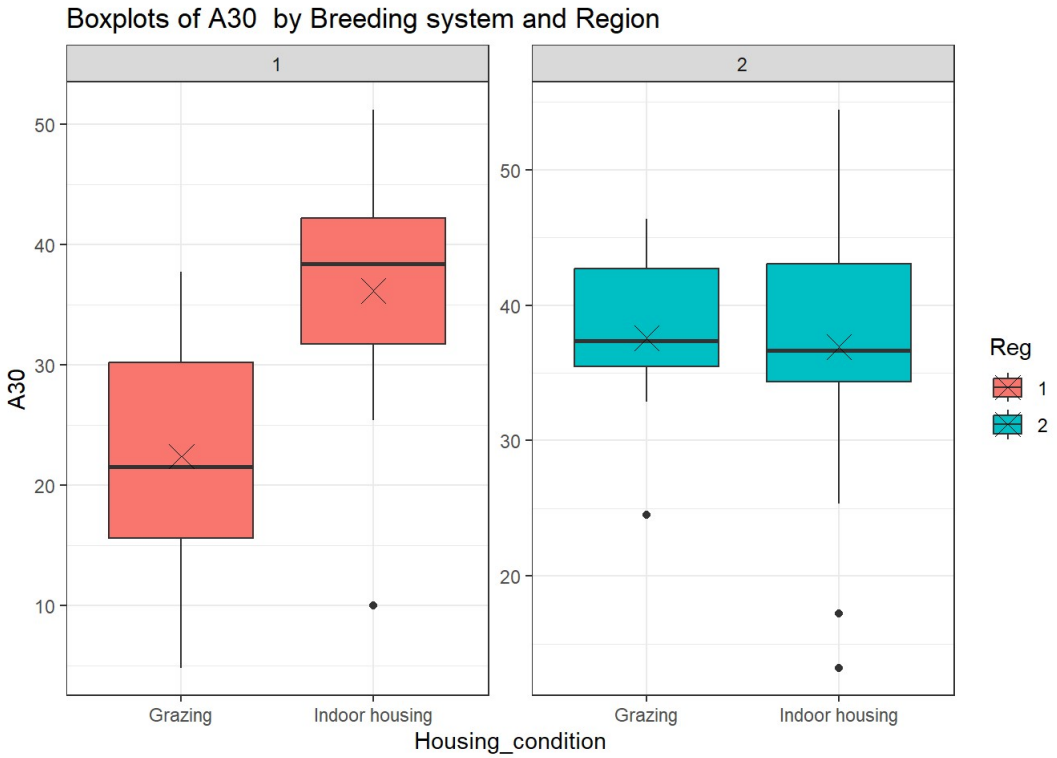

Figure 18 – Effect of Housing Condition and Region on A30.

The difference between the two regions is even more evident in *Figure 19* which represents the boxplot of the effect of the interaction between the Housing Condition and the Region on curd firmness 60 minutes from the start of the analysis.

In fact, an in-depth examination at *Figure 19* shows that, in La Dinara farm, the average A60 in the pasture is lower (19 mm) compared to the indoor condition (38 mm).

In contrast, the average A60 in the farms in Lombardy region is higher during grazing (38.4 mm) than in indoor condition (36.1 mm), although it is also clear that indoor condition demonstrates more variability.

Therefore, both A30 and A60 results showed the biggest differences considering the interaction between region and housing condition. This result highlights the significant

difference in terms of A30 and A60 in 'La Dinara' farm when comparing the two housing conditions which was not found in the other farms located in Lombardy.

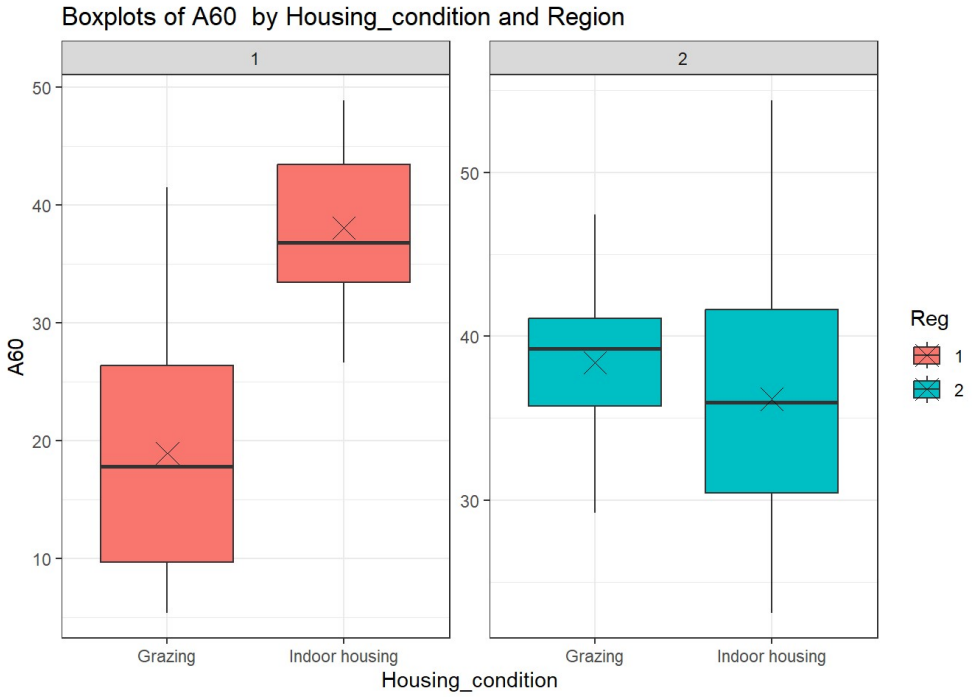

Figure 19 – Effect of Housing Condition and Region on A60.

We are also able to observe from *Figures 18* and *19* that, depending on the housing condition, the syneresis process varies between the farms in the two regions. In 'La Dinara' farm which is located in the Emilia Romagna region we can see the beginning of the syneresis process after 60 minutes in sampling during grazing (A30 22.4mm vs A60 19mm) while in the Lombardy region we notice the start of the syneresis process after 60 minutes only in the indoor condition (A30 36.9mm vs A60 36.1mm). According to Stocco, et al., 2018 the syneresis process is not much affected by milk composition and they evaluated this parameter using a modelled parameter that is the syneresis rate constant (K_{SR}). In this thesis we did not calculate the K_{SR} so we can only hypothesize that the differences found might be due to different environmental or animal factors.

5 CONCLUSIONS

In conclusion 'La Dinara' dairy goat farm in Terenzo (PR) has demonstrated excellent farm management from a genetic and milk quality point of view. It is therefore clear that even a silvopastoral family management can bring excellent results, we can also affirm this thanks to the comparison with other farming realities such as those analysed from the BIO4VERBA project.

Due of the zero average inbreeding in the BP, excellent farm management has been highlighted from the point of view of genetic variety. It is recommended to plan future mating using the ENDOG v.4.8 software and include external subjects to the farm, as genetic analyses have revealed that both AR and ΔF are growing with time in the whole population. Another suggestion is to continue using the created digital dataset and replace the paper pedigree. This change would also be beneficial for implementing breeding strategies through ENDOG v.4.8 software.

From a phenotypic point of view, analysing milk composition and quality, cheese making traits and milk coagulation properties, it was highlighted that the most significant factor is the housing condition among those analysed (Region, Farm, Housing Condition and the interaction between Region and Housing Condition). From this study, it was determined that indoor conditions produced better results than grazing in a variety of values, particularly those related to cheese-making traits maybe due to the high temperature registered this year during the grazing season.

Regarding the interaction between the region and housing condition effects, these were interesting, particularly for traits of Milk Coagulation Properties, demonstrating that in different housing conditions the farms in the two regions behave in opposite ways.

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