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Physiological approach to the study of autochthonous small ruminants breeds of Italy

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Si può fare, si può fare
Puoi prendere o lasciare
Puoi volere, puoi lottare
Fermarti e rinunciare.
Si può fare, si può fare
Puoi prendere o lasciare
Si può crescere, cambiare
Continuare a navigare…

Angelo Branduardi (1993)

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ABSTRACT

The loss of biodiversity is a worldwide issue. Many local livestock breeds are threatened by the risk of extinction, mainly due to the progressive abandonment of some of these breeds in favor of few transboundary high-performance ones. Maintaining and protecting the autochthonous breeds is pivotal to guarantee food and agriculture sustainable development and food security in the future.

In Italy, the husbandry of local goat breeds plays a multifunctional role, and expanding the knowledge of these native breeds is fundamental to allow the development of a correct strategy of conservation and improvement.

Our project has focused on the study of physiological productive, reproductive, immunological, and metabolic aspects of four local goat breeds of Northern Italy (Frisa Valtellinese, Orobica, Lariana, and Verzaschese goats), on which the information is still very limited. Moreover, we have compared the results obtained from these breeds with those from the Camosciata delle Alpi, a transboundary dairy goat breed spread all over the world.

Our results have shown the peculiarities and variabilities of the milk production of local breeds, despite their yields being lower than the cosmopolitan breed. We also investigated the polymorphism of a gene strictly related to the reproductive seasonality, showing the existence of no differences among local and cosmopolitan breeds belonging to the same macrogroup of breeds. However, when investigating different metabolic and immunological aspects, we demonstrated the greater variability among breeds and the greater rusticity of local ones. Specifically, a better quality of colostrum was found in local breeds when compared to a cosmopolitan breed, considering also the typical farming system of each of them. Moreover, evaluating the hematological parameters in different goat breeds under identical farming conditions, our study revealed a greater adaptability of local breeds to environmental stressors. Finally, this study evaluated the stress reaction to the environment during the vertical transhumance in a local breed, together with the possibility to use autochthonous breeds as biomonitoring tools, specifically for heavy metals and trace elements.

In conclusion, this project has demonstrated, with a multidisciplinary approach, the great variability of physiological adaptations existing between different breeds, and the greater rusticity and hardness enjoyed by local breeds compared to transboundary breeds.

1 INTRODUCTION

1.1 LIVESTOCK BIODIVERSITY

It is widely believed that the transformation implemented by man on the Earth's biosphere is a recent phenomenon. However, human societies shaped three-quarters of the landmass as early as 12,000 years ago, at the dawn of the Holocene Epoch. At that time, hunter-gatherer societies predominated, and farmers and shepherds were taking their first steps towards establishing sedentary societies. This had already led to the shaping of a mosaic of ecosystems of which man was a harmonious part, thanks to low-intensity and sustainable management of his surrounding environment. For centuries, the subsistence of many human societies was based on agriculture and pastoralism in different proportions and modalities depending on the pedogeological and climatic conditions of the inhabited territory (Ellis et al., 2021).

Fig.1 View of bàrech (typical fences for livestock and pasture management, whose written records of their existence date back to the 14th century, but were probably already in use in earlier times) in upper Val Corta, Tartano (SO), Italy.

The domestication process of both plants and animals, and the consequent development of agro-pastoralist societies, are a phenomenon that has not occurred in a punctual manner throughout history, but important spatio-temporal differences have contributed to the variegation of ecosystems. Indeed, the Neolithic revolution has taken place in fifteen different areas of the landmass; Fertile Crescent, China, Mesoamerica, Andes/Amazonia, Eastern United States, Sahel, tropical West Africa, Ethiopia, and New Guinea are some of the areas that have been identified as homelands of domestication events (Diamond & Bellwood, 2003; Larson et al., 2014; Mignon-Grasteau et al., 2005). Thus, a non-synchronous domestication process of the same livestock species took place, as demonstrated by archaeological evidences scattered across Eurasia that can be traced back to the ancestors of our current 'big five' domesticated animals, i.e., the cow, goat, sheep, pig and horse, plus the water buffalo (Diamond, 2002). As a result, different human societies, within a few millennia, developed more complex man-animal-environment relationships, which gave them enormous advantages over those populations left to a nomadic hunter-gatherer lifestyle. The advantages were mainly constituted by: a greater availability of food resources per area of productive land, which sustained a higher population density; the possibility of storing food, given the sedentary character of these new societies, which allowed the development of complex technology, social stratification, centralized states, and professional armies; the development of resistance and/or resilience against infectious zoonotic diseases of social domestic animals (e.g. smallpox and measles), which in contact with hunter-gatherer societies assumed epidemic trend. This allowed the first agro-pastoralist societies to exit their homelands and expand mainly along the East-West axes supplanting the weaker hunter-gatherer societies and exporting along their migrations their precious Neolithic package including domesticated plants and animals (Diamond, 1997).

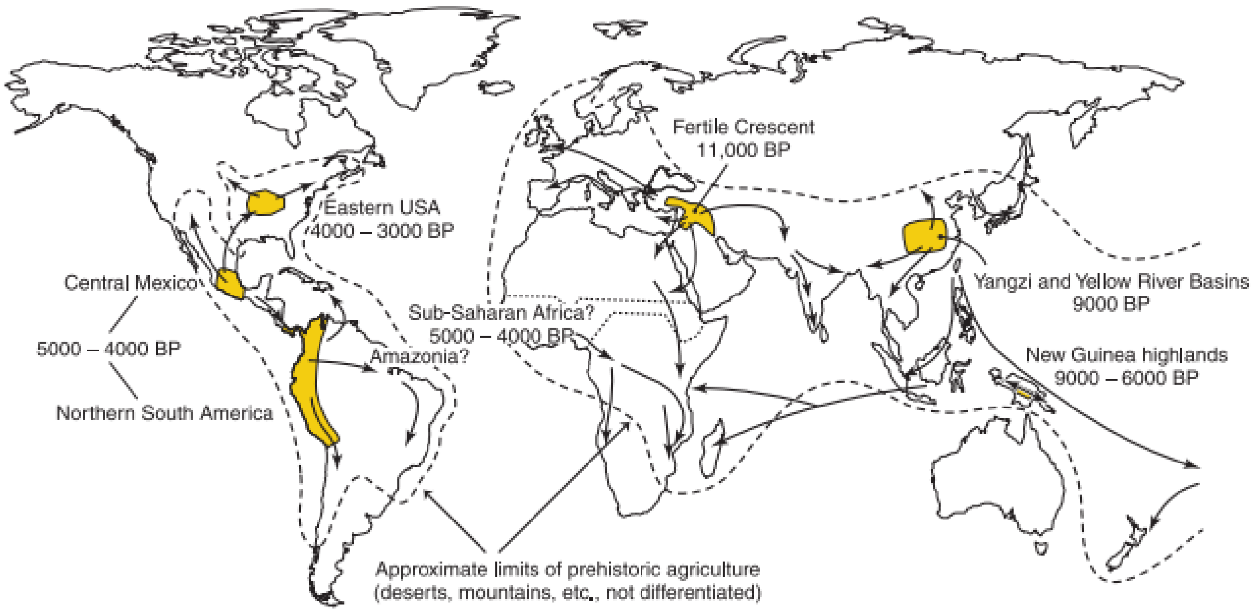

Fig.2 Archaeological map of agricultural homelands and spreads of Neolithic cultures, with approximate radiocarbon dates (Diamond & Bellwood, 2003).

From that period, a total of about 45 animal species have been domesticated by humans, as also reported in the FAO's Global Databank for Animal Genetic Resources (Domestic Animal Diversity Information System, DAD-IS) (FAO, 2023a). The reason for such a low number of domesticated species, which represent just the 0.0002% of known land animal species, is attributable to the phenological, reproductive, and behavioural characteristics of the wild ancestors of our domestic animals (Diamond, 2002). Indeed, most ancestors of domesticated animals were behaviourally preadapted to domestication, because they had some characteristics which had favoured them to get closer to humans (Zeder, 2012). However, each domesticated species has been involved in a unique human-animal relationship, shaped by diverse biological and social contingencies. Indeed, there is a remarkable variety in the ways in which animals and humans interacted, which have been formally described as 3 separate pathways: the commensal pathway, the prey pathway, and the directed pathway (Zeder, 2012). Specifically, the main ruminants domesticated species (i.e., cow, goat and sheep) followed the same prey pathway. Initially, humans approached the herds and flocks of these preys just to maximise the relative food production, managing the game wisely. The first human action involved feeding mainly on young males or on females that were no longer fertile, later progressing with a more targeted management of reproduction and also of the diet of these animals. Then, the relationship proceeded with the formation of real herds, under the control of a shepherd, made up of animals characterised by having a docile disposition that allowed an extensive farming system. Finally, there was the transition to the actual captive animal control and intensive breeding. The domestication process in this case was not voluntary on the part of man, who initiated it just to make a food resource more efficient (Larson & Fuller, 2014).

Fig. 3 Wall painting from the tomb of Nebarunun, Thebes, Egypt, c1350 BC showing him inspecting his cattle herds.

As a result of the domestication process, human populations have gradually, over millennia, brought new prototypes of domesticated animals along on their migrations. Specifically, the main livestock ruminants (i.e., cattle, goat and sheep) were independently introduced to Europe from their homelands of origin following two major routes: the first was along the Mediterranean coast, the second along the Danube (Gkiasta et al., 2003). Thus, the newly domesticated animals, being bred in different pedogeographical, climatic and cultural contexts, and undergoing introgression, natural and artificial selection processes, gave rise to the generation of a large number of different breeds specifically adapted to different conditions and productions. This is what today represents the so-called livestock biodiversity, also referred to as animal genetic resources (AnGR) (Hall, 2004). As reported by Prof. Irene Hoffmann, which has been the Secretary of the Food and Agriculture Organization of the United Nation's (FAO) Commission on Genetic Resources for Food and Agriculture from 2015 till June 2023, the concept of breed is quite ambiguous, because is linked to a cultural concept rather than to a physical one, and for this reason, it differs from a country to another (Hoffmann, 2010). However, FAO, thanks to its magnificent work of collecting data from more than 160 countries in 2007 (FAO, 2007) and nearly 130 in 2015 (FAO, 2015), has compiled a list of the world's livestock breeds and included each of them in the Domestic Animal Diversity Information System (DAD-IS) (FAO, 2023a). According to the Second Report on the State of the World's Animal Genetic Resources by FAO, a total of 8774 breeds have been identified within the 38 livestock species considered. Of this total, 7718 (88%) are the local or autochthonous ones (i.e., breeds present in only one country), while only 1056 (12%) are classified as transboundary or cosmopolitan breeds (i.e., breeds present in more than one country) (FAO, 2015). However, the greater part of the local breeds is considered at risk (already extinct or currently at-risk).

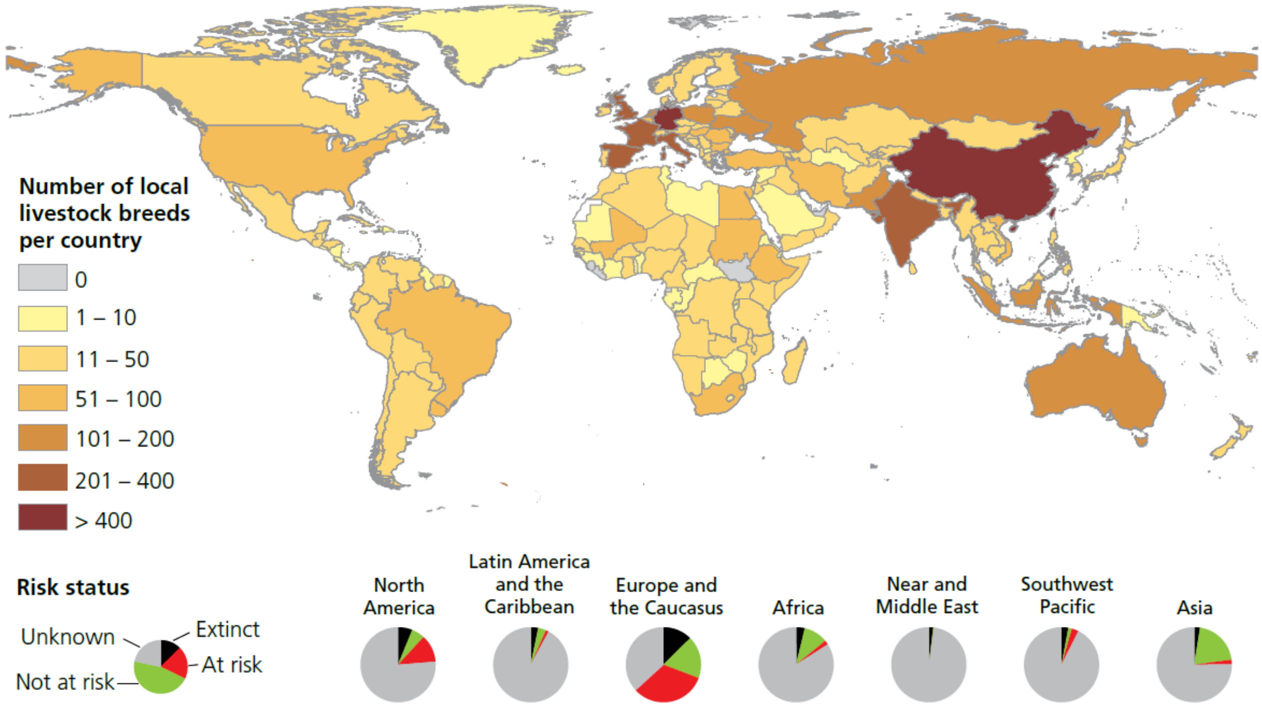

Fig. 4 Livestock diversity around the world (FAO, 2015).

This negative trend, which almost exclusively afflicts autochthonous breeds while sparing the cosmopolitan ones, has been taking place for the past 250 years, and has led to extensive changes never before seen in the history of livestock biodiversity. In particular, the industrial revolution with new means of transport that allowed for the movement of livestock over long distances, followed by the spread of artificial insemination as a method of breaking down genetic isolation, catalysed this change (FAO, 2007). The dichotomy of local vs. transboundary breeds was and is linked to the intrinsic breeding characteristics of each of the two, which clashes directly with the production needs, especially in developed and developing countries. In fact, while local breeds are characterised by medium-low external input systems, with the provision of a wide range of ecosystem services and low-output products, cosmopolitan breeds are generally able to produce a single primary product (e.g., milk or meat) more efficiently at the cost of higher levels of external inputs. For these reasons, transboundary breeds are designed to perform to their full potential in very well-monitored and controlled systems, i.e., in intensive farming regimes. In contrast, autochthonous breeds, are traditionally reared in semi-extensive to extensive regimes, exploiting what natural ecosystems offer and thus obtaining food products in a self-sufficiency regime (FAO, 2003, 2007). Each of the two types of breeds is therefore adapted to different farming systems, and is used efficiently in different contexts. In fact, a long-term global strategy for the future should consists in improving the sustainability of cosmopolitan breeds farming and, at the same time, using local ones to produce and supply different ecosystem services, acting as reservoir of unique genes (Pulina et al., 2017).

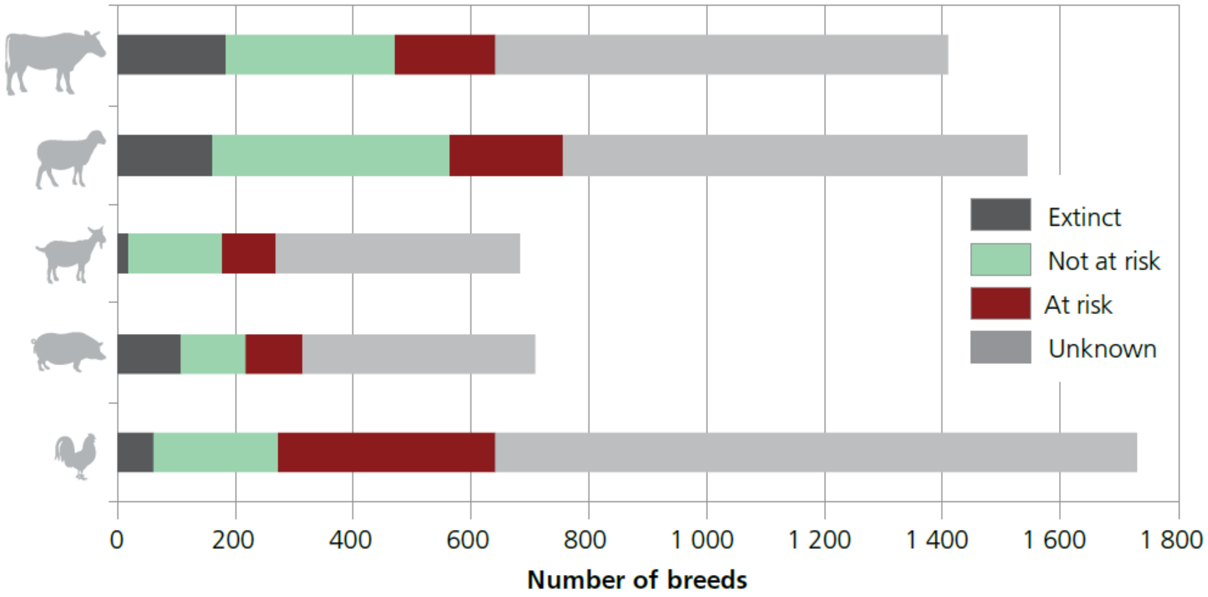

Fig. 5 Status of the world's livestock breeds (FAO, 2015).

Thus, aware of this context, what is the significance of livestock biodiversity in terms of AnGR? The answer is straightforward. It represents the raw material on which genetic improvement projects can be implemented for the selection of animals capable of coping with climate change and the continuing growth of the world population, ensuring agriculture and food production sustainable development (FAO, 2019). Indeed, AnGr is a reservoir of genes with a huge potential for facing the future challenges, in particular when considering the breed-specific adaptive traits of local breeds (Leroy et al., 2018). Thus, the best definition for biodiversity for food and agriculture is "the variety and variability of animals, plants and micro-organisms at the genetic, species and ecosystem levels that sustain the ecosystem structures, functions and processes in and around production systems, and that provide food and non-food agricultural products", according to FAO (FAO, 1999). However, livestock biodiversity, as well as domesticated plant biodiversity, result from the undeliberate and deliberate action of humans. That is the main characteristic that distinguishes that type of biodiversity from the natural one, which does not require the constant human intervention to be maintained. Indeed, agricultural biodiversity has evolved with humankind, and is closely interdependent from its declinations such as economy, societies, cultures and knowledge systems (Hoffmann, 2010).

The first steps toward recognition of the importance of biodiversity was moved in 1992 with the adoption by 168 countries of the Convention on Biological Diversity during the United Nations Conference on Environment and Development (i.e., Earth Summit) in Rio. According to this document, biodiversity is “the variability among living organisms from all sources including, *inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems” (Glowka et al., 1994). Moreover, the Convention enshrined in law the need to preserve biodiversity at the level of the ecosystem, species, and genes, defining its conservation as "a common concern of humankind" and recognizing its fundamental environmental, economic, and social role (Glowka et al., 1994). Following this, in 1995, as an evolution of the Commission on Plant Genetic Resources, the FAO Commission on Genetic Resources for Food and Agriculture was officially established, consisting of 178 member countries plus European Union. It was the first intergovernmental body whose exclusive task was to develop plans for the conservation, sustainable use and fair and equitable sharing between countries of biodiversity of relevance to food and agriculture (Resolution 3/95). Thanks to the hard work of this committee, the first two reports on the state of AnGR were drafted (FAO, 2007, 2015), as well as the drafting and adoption by 109 countries of the Global Plan of Action for Animal Genetic Resources (Commission on Genetic Resources for Food and Agriculture (FAO), 2007). This document is structured into 23 strategic priorities for action, which are in turn grouped by priority area. Specifically, within the first area 'Characterization, Inventory and Monitoring of Trends and Associated Risks', Strategic Priority 2 deals with 'Develop International Technical Standards and Protocols for characterization, inventory, and monitoring of trends and associated risks', and therefore foresees that each country must commit itself to phenotypically characterizing its AnGR, using, among others, methods for the qualitative-quantitative assessment of production, nutrient use, functional, and economic traits. All this, also taking into account environmental, socio-economic, and cultural factors relating to the management of livestock biodiversity (Commission on Genetic Resources for Food and Agriculture (FAO), 2007).

Therefore, it is up to each country, for ethical reasons as well as international agreements, to protect its livestock biodiversity in the best possible way. Each country adhering to the Global Plan of Action for Animal Genetic Resources has the task of drawing up National strategies and action plan, and on the basis of that to take actions aimed at the conservation, sustainable use, and fair and equitable sharing of AnGR (Commission on Genetic Resources for Food and Agriculture (FAO), 2007). In Italy, this plan was drafted in 2013. Before the adoption of this plan, several actions had been already carried out, for example with the establishment of herdbooks to monitor the local breeds consistency and to help farmers in pursuing conservation and genetic improvement programs. In 2019, the latest Progress Report on the Implementation of the Global Plan of Action for Animal Genetic Resources (covering the period from 2014 to 2019) was published by Italy. From its analysis, a stable but potentially critical situation emerges. In fact, despite the government's economic support to breeders has been very efficient in counteracting the erosion of AnGR, any discontinuity in the disbursement of these public funds could put the entire plan at risk. Indeed, the profitability of the autochthonous breeds’ farming is achieved not only through the sale of the products, but also through contributions from the European Agricultural Fund for Rural Development (EAFRD) for the implementation of rural development programs (RDP). For instance, the RDP 2014-2020 of the Lombardy region (North Italy), extended until 31 December 2022, envisaged for local small ruminants’ breeders a premium for those rearing local breeds of Lombardy. The farms of each breed must be located in the breed's relative 'areas of diffusion', established by the law itself and based on historical and traditional farms. From 1 January 2023, however, the new Common Agricultural Policy 2023-2027 came into force, which changed the amounts paid and added new conditionality for the farmers. However, in addition to contributions, in Italy, a major role in the conservation of local breeds is certainly to be attributed to Breeders' Organizations and other private associations such as RARE (Razze Autoctone a Rischio Estinzione, i.e., Autochthonous breeds at risk of extinction). In fact, in many cases the success of keeping a local breed lies in the ability of its breeders to valorize and create a niche product, so as to be able to increase the selling price to a sought-after public. In this way, the breeder is remunerated for the added value of the product, which lies behind its mere quality, i.e., above all its ecological, social, and cultural value.

Taking into account what mentioned above, it must be admitted that there is still much to be done, and not only in the Italian state, to protect AnGR. Many local breeds have already gone extinct or are functionally extinct or are in great danger, such as the Bormina goat or the Saltasassi sheep. As stated in Strategic Priority 2 of the Global Plan of Action for Animal Genetic Resources, the study of the physiological characteristics of local breeds is a key point for a successful conservation and implementation policy.

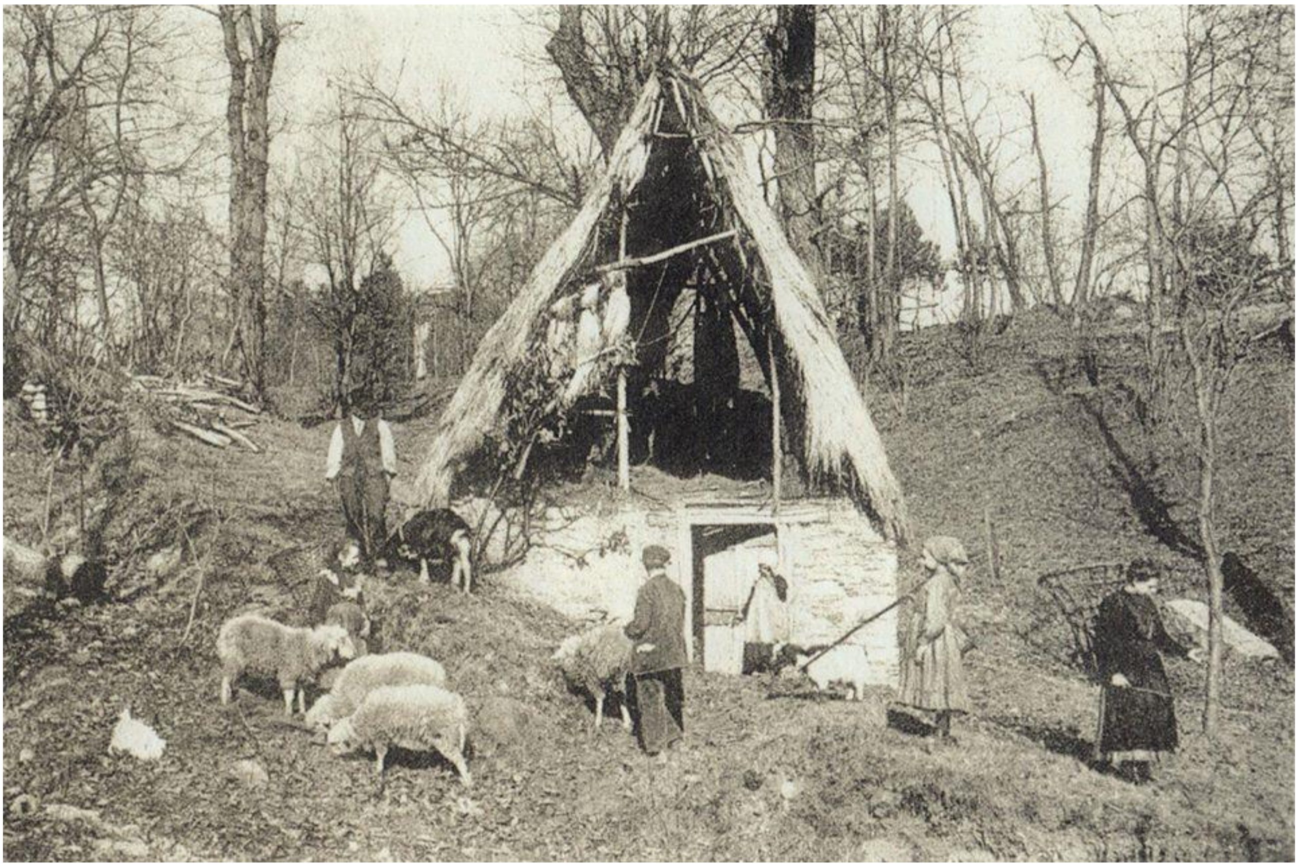

Fig. 6 Saltasassi sheep near Lago Maggiore in the vicinity of Mount Mottarone, c. 1940.

1.2 MULTIFUNCTIONAL ROLE OF LOCAL LIVESTOCK BREEDS

As previously mentioned, the best definition for agrobiodiversity, is "the variety and variability of animals, plants and micro-organisms at the genetic, species and ecosystem levels that sustain the ecosystem structures, functions and processes in and around production systems, and that provide food and non-food agricultural products" (FAO, 1999). The second part of this sentence directly refers to the concept of "ecosystem services" (ECs), which are the broad range of benefits that humans obtain from ecosystems. In the specific case, the ECs are those supplied by the agrobiodiversity, when interacting with natural ecosystems and their processes (Millennium Ecosystem Assessment (Program), 2005). Specifically, the main ways in which livestock biodiversity interacts with natural ecosystems are three, namely through the transformation of human non-edible material into food and non-food products, through the action of grazing, browsing, trampling, defecation, and urination, and through their movement in response to spatio-temporal variations in available resources (FAO, 2014). This leads to the production of a series of ECs, which have been formally classified in different categories based on the type of benefit provided to people. The most recent description of ECs by FAO provides 3 categories: (i) provisioning services, (ii) regulating, supporting and habitat services, and (iii) cultural and amenity services (FAO, 2020). Moreover, it is often believed that more diverse biological communities, both at inter-species and intra-species levels, are able to supply ECs in a more effective and resilient way, thanks to the greater ability to adapt to any environmental variation. However, when not well or sustainably managed, agrobiodiversity could also be responsible for the supply of ecosystem disservices rather than services, for instance, when overgrazing is practiced (FAO, 2020). For these reasons, it is of pivotal importance to improve the responsible and sustainable management of the AnGR in order to derive the greatest possible benefit from the ECs produced by them.

To give an example, in many Italian alpine and prealpine marginal areas, the semi-extensive breeding of double purpose autochthonous small ruminants represents an important source of income. However, it doesn't end with the mere production and sale of dairy products and meat (and rarely wool or leather); the grazing action of these animals is involved in supplying numerous ECs, such as maintenance of soil quality, habitat provisioning, climate and natural-hazard regulation, and landscape maintenance (Battaglini et al., 2014). In addition, the pasture exploitation also has socio-economic implications with its positive effect on tourism (Scarpa et al., 2011) and by allowing the maintenance of traditional agro-pastoral practices and the production of traditional products, which are part of the cultural heritage (Di Trana et al., 2015). This type of low input animal husbandry therefore has a multifunctional role in providing different ECs, which is responsible for the added value of its products (Cassandro, 2015).

1.2.1 PROVISIONING SERVICES

As previously mentioned, the first category of the ECs classification is represented by the provisioning services. That are the ECs provided by the mere agrobiodiversity production of food (e.g., meat, milk, eggs) and non-food products (e.g., fibre, skin, dung, motive power). The amount and the variety of material products supplied is strictly dependent on the breed and farming system used. Usually, single-purpose breeds are those artificially selected in the last centuries and that are able to give the best performance in controlled environments, such as those of the intensive farming system. However, as previously mentioned, this type of farming requires high external input to get to high output, and is often associated with other ecosystem disservices, if not well balances and controlled from a sustainability point of view. On the other hand, the farming of multipurpose local breeds is usually associated with low external input mixed or grassland systems, thanks to the high adaptability of these animals to the specific ecosystem where they are traditionally bred. Also in this case, if well managed, these breeds are able to provide a variety of other material and non-material benefits in addition to subsistence (FAO, 2020).

1.2.2 REGULATING, SUPPORTING AND HABITAT SERVICES

This type of ECs is supplied by agrobiodiversity to people via regulation and support of the natural ecosystem processes and via maintenance and provision of wild life habitats. Thus, these ECs are comprehensive of all those actions that are indirectly connected with the farming of livestock and that are responsible for improving the ecosystems, making them less polluted, more functional and resilient to change. A typical example of those ECs is the natural-hazard regulation (e.g., wild fires, avalanches, floods, landslides) (FAO, 2020; Leroy et al., 2018). Many studies have been conducted evaluating the ability of different domestic ruminants, and in particular goats, to prevent the spread of wild fires thanks their grazing and/or browsing action (Lovreglio et al., 2014). Also in this case, local breeds cover a pivotal role, being the most adapted to the territory, and usually able to thrive on low quality feed.

Fig. 7 A Boer goat flock implemented by Fire Department in San Rafael, California (USA) for wildfire prevention.

Moreover, the grazing/browsing action can also have a role in the weed control and biomass residue management, and a link with the pollination service has also been hypothesized (Hopwood et al., 2015). Other regulating ECs are the soil erosion prevention and its quality maintenance: the positive provision of these services by domesticated livestock is strictly linked to the grazing practices adopted by the shepherds, in terms of grazing density, pasture rotation, species employed, etc.. Finally, the provision of habitat is another important ECs for the maintenance of specific wild species. Indeed, in those co-evolved landscapes, AnGR cover a pivotal role in maintaining the life cycle of both local wild flora and fauna (FAO, 2020; Leroy et al., 2018). In particular, avifauna is one of the most affected class of species by the progressive human depopulation in the Alps. Many birds, which find their ideal habitat in open grasslands, are gradually becoming extinct due to the disappearance of alpine pasture areas, as the traditional system of semi-extensive breeding of local breeds of cows, small ruminants, and equids with annual vertical transhumance is becoming less and less practised (Bazzi et al., 2015; Laiolo et al., 2004; Parolo et al., 2011).

1.2.3 CULTURAL AND AMENITY SERVICES

Livestock biodiversity has co-evolved with humans and all their declination, including economy, societies, cultures and knowledge systems (Hoffmann, 2010). For this reason, nowadays breeds can be considered as a manifestation of both historical and contemporary social interactions, and therefore, as part of our cultural heritage. That is particularly true when considering local breeds, which often provide also non-material benefits, in addition to subsistence (Hall, 2019). The cultural services are the most difficult to be assessed, even if some proposals for the evaluation of the cultural value of livestock biodiversity have been done (G. C. Gandini & Villa, 2003). Usually, the aspect most considered when evaluating the cultural services provided by agrobiodiversity is that related to the guardianship of local gastronomic traditions, thus neglecting all that concerns aesthetic, educational, spiritual, recreational aspects linked to a given breed and the related human-animal relationship (FAO, 2020). An emblematic example is the 'bataille de reines' held every year in Valle d'Aosta. It is an event whose focus is the Valdostana breed of cows, which has a strong social/folkloric meaning. Moreover, some local breeds are also involved in the determination of the so-called “sense of place” associated with a given location (Hausmann et al., 2016), as in the case of the famous North Ronaldsay sheep, which is characteristic of the North Ronaldsay island, and concurred to shape its landscape. Hence, the cultural significance of a particular breed can be extended to consider the knowledge systems associated with it, including not only production methods and culinary traditions but also religious, folkloristic, artistic rituals, and more. All this is therefore threatened by the progressive abandonment of local breeds and related farming systems, resulting in the loss of a true cultural heritage. Therefore, in addition to the previously mentioned reasons, making plans for the conservation and maintenance of AnGR is also fundamental to preserve part of human history.

Fig. 8 Bataille de reines, 1987, vallon de Vertosan, Avise, (AO), Italy.

1.3 GOAT HISTORY AND LOMBARDY BREEDS

The goat has a very ancient familiarity with humankind, being among the first animals to have been domesticated. The food resources provided by goat have always been valuable to human populations that have used them for sustenance since the Holocene epoch. Indeed, the fundamental role of the goat within numerous ancient societies is also demonstrated in Greek literature, where we find the mythological figure of Amaltheia, the goat that suckled the baby Jupiter inside a cave in Crete (Evelyn-White, 1914).

Fig. 9 "The Goat Amalthea with the Infant Jupiter and a Faun" - Gian Lorenzo Bernini 1609-1615.

Capra hircus Linnaeus, 1758, the domestic goat, belongs to the order Artiodactyla, family Bovidae, which means that males of this species have always two or more unbranched horns that do not shed, and females usually have too. The subfamily is Caprinae. This Bovidae subfamily differs from the others in that its members have evolved by specifically adapting to particularly harsh environments, being that the ecological niche of the Great Plains had already been entirely occupied by Cervidae species at the time. The maximum diversity of the Caprinae was reached at the end of the last Ice Age, when this subfamily colonized desert, alpine, and tundra environments (Geist, 1984). The evolutionary history of goats and sheep, or rather, of their respective wild ancestors, i.e., the wild bezoar ibex and the Eastern mouflon, began to diverge when the goat ancestors started to adapt to more inaccessible environments and/or with low quality-quantitative food availability, while the latter for terrains such as foothills, which were less steep and with different feeding possibilities. All these led members of the *Capra* genus to develop a greater ability to climb rocks (as denoted, for example, in alpine breeds) (Clutton-Brock, 1999).

Fig.10 Orobica, Frisa and Verzaschese goats during the alpine pasture in 2022 summer, Preda Rossa Val Masino (SO), Italy.

The *Capra* genus then spread to Africa and Eurasia, never reaching America, due to the failure to cross the Bering Strait during the Pleistocene, when there was still the Bering land Bridge that allowed the passage from the Old to the New World (Clutton-Brock, 1999). The modern domestic goat (*Capra hircus*) as we know it today, among its more than 600 breeds registered in DAD-IS (FAO, 2023a), has origins dating back to around 11000 years BP, in the mountains of the Fertile Crescent. It was among the first animal species to be domesticated by humans, demonstrating remarkable adaptability and versatility. The wild population of goat ancestors with which Neolithic man came face to face at the beginning of the domestication process were the precursors of the modern wild bezoar (*Capra aegagrus*) (Zeder, 2008). Genetic studies have shown that the main target at the beginning of the domestication process was genes related to behaviour, which resulted in more docile animals. As previously mentioned, the first prototype of the domesticated goat than adapted to new environments following the migrations of human populations. During this long process that took thousands of years, several introgression events occurred. In particular, the introgression event from a West Caucasian tur-like species led to the acquisition by the modern goat population of genes related to pathogen resistance. Finally, the spatial and temporal distancing from the domestication centre of the new goat populations, caused genetic divergence among them, leading to the differentiation of specific breeds within 3 different clusters: the Asian cluster, the European cluster, and the African cluster (Zheng et al., 2020).

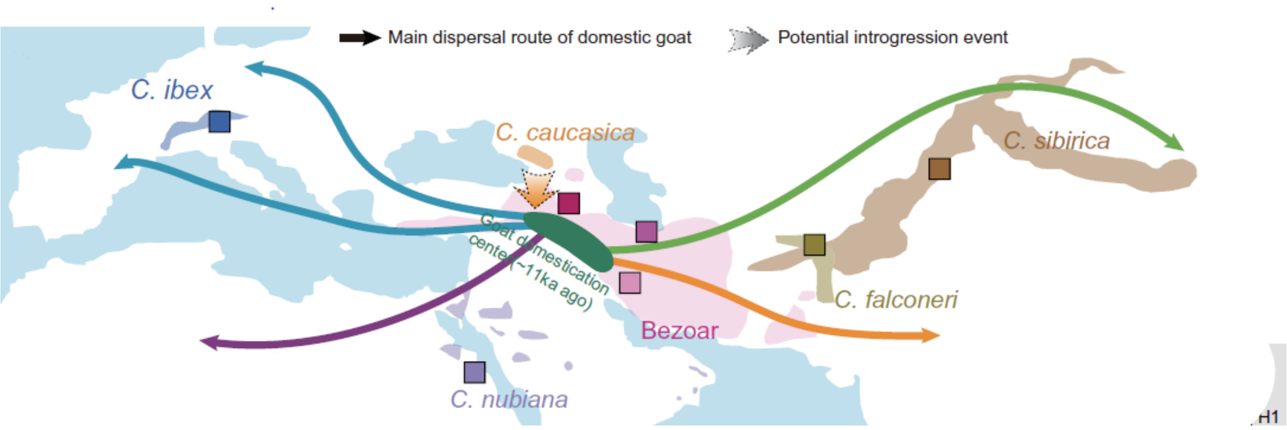

Fig.11 Geographic distribution of the wild Capra species and the dispersal routes of domestic goats out of their domestication areas (Zheng et al., 2020).

Nowadays the actual world goat population is about 1.1 billion of heads. According to FAO, over 90% of goats are found in developing countries, mainly distributed in Africa and Asia (FAO, 2023b).

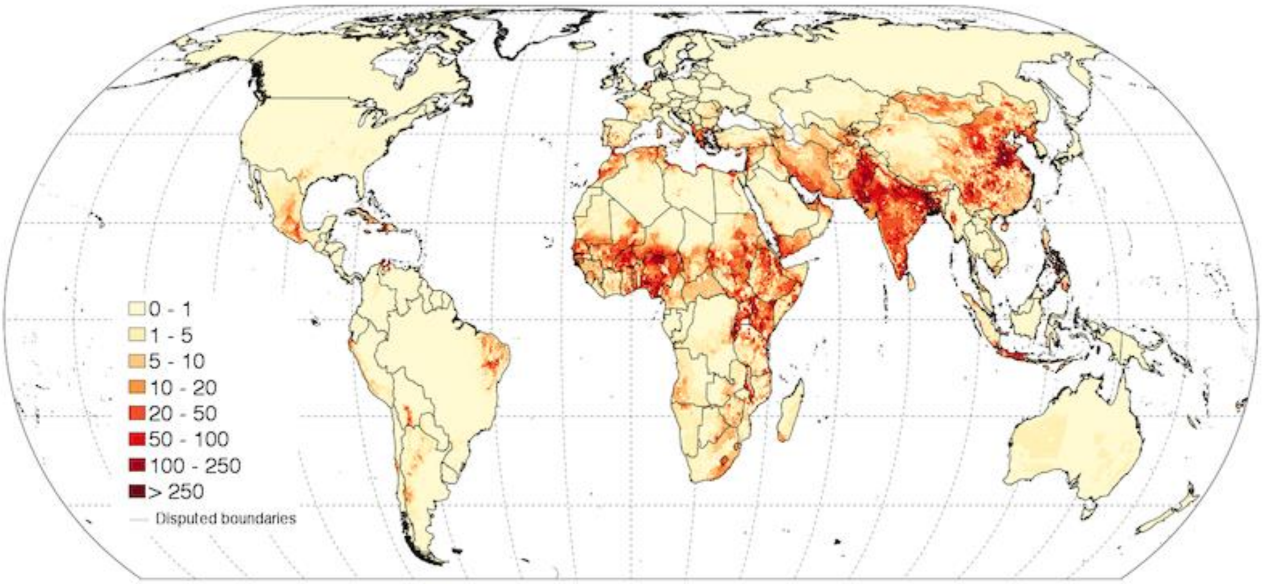

Fig. 12 Estimated worldwide distribution and density of goat heads per square kilometer in 2010 (Gilbert et al., 2018).

The Second Report on AnGR for food and agriculture by FAO, recognized the existence of 576 local goat breeds, of which nearly 40% from Europe, followed by Asia and Africa, while 86 transboundary breeds are reported, and Europe is also the leader in this category (FAO, 2015). Among the most bred transboundary breeds, there are different dairy goats, such as the Alpine, the Anglo-Nubian, the Toggenburg, and the Saanen; Boer and Kiko goats are the most important meat purpose breeds; finally, Angora and Kashmir goats are the most famous fiber-producing breeds (Ferro et al., 2017).

Different studies have reported the specific characteristics of some of the local breeds, which confer them advantages, thanks to the adaptation to specific environments/conditions (FAO, 2015; Simões & Gutiérrez, 2017; Taberlet et al., 2008). Specifically, according to FAO, 62 goat breeds have been reported to be particularly adapted to the mountainous environment, 30 to be heat-tolerant, 7 humidity-tolerant, 20 drought-tolerant, 14 cold-tolerant, 20 adapted to water scarcity, and 11 to extreme diets (FAO, 2015). Thus, the physiological study of these interesting characteristics of local breeds, could lead to important development in the zootechnical field for the future. For example, the specific strategies of adaptation to feed scarcity, which is a current problem considering the ongoing worldwide desertification process (Huang et al., 2020), are being investigated. Mainly four different strategies of adaptation have been identified: to possess a low metabolic requirement compared to other breeds of the same species, to possess the ability to reduce it in extreme conditions, higher digestive efficiency, mainly through the ability to exploit all the potential energy from a high-fibre feed, finally, to have the ability to deposit reserves of nutrients, usually represented by fat deposits (FAO, 2015). As for the third adaptation strategy, an example of a representative breed is the Black Bedouin or Badali goat from Lebanon and Jordan. It is reared for both meat and milk production, and it is used to graze on poor-quality and high-fibre feed. It has been demonstrated that this breed has significantly higher digestive efficiency on poor types of forage than a transboundary breed such as the Saanen (Silanikove et al., 1993). The physiological reason for these differences lies in two aspects: the first is a longer mean retention time of feed in the rumen, which probably could be ascribed to a more efficient mechanism of retention; the second is the higher microbial activity of the rumen microbiota, which is a consequence of a higher ability to maintain the ruminal pH in the optimum range for the microbiota even after feeding events. This causes a higher production of volatile fatty acids with a specific composition which confers more energy to the Baladi goat when compared to the Saanen. The ability to cope with the pH reduction is probably due to a higher absorption rate of the volatile fatty acids from the ruminal wall (Silanikove et al., 1993). However, the adaptation to feed scarcity is not the only one which has been investigated. For instance, other studies have shown an adaptive advantage of local breeds over cosmopolitan ones concerning disease resistance and/or resilience, and, specifically, in the case of small ruminants, to gastro-intestinal parasitosis (González et al., 2012).

In Italy, studies on these topics are still fragmentary, although they are gradually increasing, also thanks to the funding provided in this area of research by the Rural Development Plans (PSR) to support the development of rural areas (European Commission, 2023). However, to understand the current situation of the goat livestock sector in Italy, it is necessary to investigate into the historical significance and role that goats have played throughout the entirety of the Italian peninsula's human history. Indeed, despite the fact that during the Roman Empire the goat was held in high esteem for its products that were highly valued by the societies of the time, beginning with the barbarian invasions, and continuing into the late Middle Ages, goat farming in Italy suffered a severe contraction. Then, during the Fascist period, laws that limited and discouraged goat breeding, which was unjustly associated with environmental degradation, caused a further reduction in the numbers of many goat breeds, which often reached a negative peak during the economic boom of the 1970s due to the depopulation of rural and mountainous areas. It is only in the last few decades that the goat as a livestock animal is being revalued, but often taking into consideration only cosmopolitan breeds. Today, Italy has numerous autochthonous goat breeds. According to DAD-IS database in Italy there are 45 different goat breeds, and just 4 of them are classified as transboundary (Camosciata delle Alpi, Maltese, Murciana, and Saanen) (FAO, 2023a). All the others are local breeds and, except for the Rustica di Calabria and Aspromonte, are considered at risk (FAO, 2023a). ASSO.NA.PA. (National Association of Sheep Breeding) is the Italian Association for the protection of the national livestock heritage of sheep and goat. It is responsible for maintaining the genealogical herd books for the cosmopolitan goat breeds (Alpine, Saanen, and Sarda) for their genetic improvement. In addition, it is also in charge of the ex-anagraphic registers, now also genealogical herd books, for the local goat breeds with limited distribution (n=37) and for the foreign ones (n=1). The purpose of ex-registers is to conserve populations with a focus on maintaining their genetic variability while promoting, where possible, their economic valorisation (*ASSONAPA Associazione Nazionale Della Pastorizia*, 2023).

Today, in many Italian alpine and prealpine marginal areas, the semi-extensive breeding of multipurpose autochthonous small ruminants represents an important source of income for small to medium-sized farms. Nevertheless, in the Alps, during the last decades a negative trend regarding the number of small ruminants' farms has been outlined, while the number of heads per farm has greatly increased. That is the result of what was stated above: a gradual transition from the semi-extensive breeding of local breeds, to an intensive production system that utilizes just a few high-performance breeds (FAO, 2019). In fact, the main goat breeds present in Northern Italy are the Saanen and the Alpine, while the local breeds represent a minority (Sandrucci et al., 2019). As previously mentioned, the transboundary breeds have often undergone a selection program that has led to high-performance animals with low intra- and inter-breed genetic variability (Biscarini et al., 2015). By focusing on the quantitative production traits during selection, as has happened in the cattle sector, other aspects, such as production quality, reproduction, longevity, disease resistance, and adaptation to poor forage, tend to get worse or even disappear completely (Taberlet et al., 2008). To give an example, many Italian studies (Claps et al., 2014, 2016; Currò et al., 2019, 2020; Manuelian et al., 2020; Tripaldi et al., 1998; Vacca, Stocco, Dettori, Pira, et al., 2018; Vacca, Stocco, Dettori, Summer, et al., 2018) have assessed the dairy products' quali-quantitative characteristics of different Mediterranean autochthonous goat breeds versus Saanen, Camosciata Alpina, and Maltese goats. The studies have highlighted a higher milk yield of the cosmopolitan breeds, but a lower milk nutritional quality (Currò et al., 2019, 2020; Manuelian et al., 2020; Tripaldi et al., 1998; Vacca, Stocco, Dettori, Pira, et al., 2018). Also, the coagulative aptitude and nutritional value of cheeses were higher in the Mediterranean breeds (Currò et al., 2020; Vacca, Stocco, Dettori, Pira, et al., 2018; Vacca, Stocco, Dettori, Summer, et al., 2018), and the colostrum from Garganica goats versus Saanen or Maltese have shown interesting oligosaccharides concentrations (Claps et al., 2014, 2016). Regarding disease resistance, there are many worldwide studies testifying the natural resistance or resilience of local breeds to diseases endemic to their area of origin, especially concerning parasites. That is often a result of both a long co-evolution between pathogen agent and host, and a greater exposure to harmful microorganisms than cosmopolitan breeds (Alberti et al., 2012; Baker et al., 1998; Piedrafita et al., 2010; Serradilla, 2001). Different studies on Italian local goat breeds have also demonstrated this thesis (Alberti et al., 2014; Zanzani et al., 2019). In addition, the high intra-breed genetic variability which characterized local breeds, makes it more likely to find genetic variants among their populations which confers disease resistance, such as in the case of resistance to Scrapie of some Sicilian autochthonous goat breeds (Vitale et al., 2016). Moreover, also from a metabolic point of view, we can assess as well the greater adaptive capacity of local breeds compared to transboundary ones in relation to the local environment (Manuelian et al., 2020).

Taking into account what has been mentioned so far, and consulting the scientific literature available to date, there is a lack of investigations regarding the local goat breeds of Lombardy. So far, several genetic studies have been conducted, particularly focused on the casein complex polymorphism and genetic variability (Ajmone-Marsan et al., 2001; Brambilla et al., 2003; Caroli et al., 2001, 2006; Chessa et al., 2005; Chiatti et al., 2005, 2007; Cortellari et al., 2021; Crepaldi et al., 2001; Meggiolaro et al., 2003; Nicoloso et al., 2015). Additionally, there have been investigations into parasitic resistance/resilience (Alberti et al., 2014; Zanzani et al., 2020) and the quality of select traditional products (Paleari et al., 2008) related to these breeds. However, a knowledge gap still exists when it comes to various physiological aspects, including production, reproduction, metabolism, and immunity. This information is fundamental in order to establish a correct management strategy that could guarantee the protection and improvement of the local breeds.

In Lombardy, the goat breeds that have been recognized with the institution of a herd book for breeds with limited distribution are 5: Bionda dell'Adamello, Frisa or Frontalsca, Lariana or di Livo, Orobica or di Val Gerola, and Nera di Verzasca or Verzaschese. For the aim of this thesis, only breeds bred in the Central-Western Alps and Prealps of Lombardy were taken into account. For this reason, Bionda delll'Adamello was not investigated. Following, a description of the local breeds that have been studied in this project.

The Frisa or Frontalasca goat is native to the Rezzalo valley (in the Frontale municipality, Italy) and probably derives from the Grisons Striped Swiss breed (Rubino, 1993). It is mainly reared in Valtellina, Malenco Valley, Masino Valley, Valchiavenna, Valcamonica, Bergamo Valleys and in the Lario area (ARAL (Associazione Regionale Allevatori Lombardia), n.d.). Genetically it has been proven to be highly related to the Bionda dell'Adamello goat (Aimone-Marsan et al., 1999; Ajmone-Marsan et al., 2001; Crepaldi et al., 2001). The Anagraphic Registry was activated in 1997, and in 2022 the registered population was 2048 heads in 50 farms (FAO, 2023a). The farming system is the traditional one, comprising of indoor housing in winter with a hay-based diet, natural grazing in spring and autumn, and vertical transhumance in summer, even if a semi-wild grazing system from spring to autumn is also a common practice (ARAL (Associazione Regionale Allevatori Lombardia), n.d.). It's a double-purpose breed with a good meat production of kids that are slaughtered at 38 days with an average weight of 12.5 kg (Crepaldi et al., 2002). The traditional Valchiavenna goat Violino derives from the seasoning of legs and shoulders of adult goats (ARAL (Associazione Regionale Allevatori Lombardia), n.d.; Paleari et al., 2008). The average milk yield is 311 L with 3.39% fat and 3.29% protein content (AIA (Associazione Italiana Allevatori), 2020), and it is mainly used for cheese production. The most relevant morphologic characteristics are the black coat (short or medium length hair) with white stripes on both sides of the head till the ears, white on the ventral part of the abdomen, on the limb distal extremities and under the tail, and the ibex type horns (even if polled subjects may be seen) (Crepaldi et al., 2001). It is a large size goat well proportionated and with a strong constitution that allows it also the exploitation of the most difficult pastures (ARAL (Associazione Regionale Allevatori Lombardia), n.d.).

Fig. 13 Frisa goat during the spring pasture in valley, Cosio Valtellina (SO), Italy.

Lariana or di Livo goat is mainly reared in the Livo Valley and Western Lario, and its origin is unknown (ARAL (Associazione Regionale Allevatori Lombardia), n.d.). It is classified as a traditional or primary population because of its highly phenotypic polymorphism (Crepaldi et al., 1999); however, genetically it is highly related to the Verzaschese (which is reared in valleys adjacent to those of the Lariana) and also, in a lesser extent, to the Bionda dell'Adamello and Frisa Valtellinese (Ajmone-Marsan et al., 2001). In fact, the somatic originality is not supposed to be related to genomic divergence (Crepaldi et al., 2001). The Anagraphic Registry was activated in 2001, and in 2022 the registered population was 1901 heads in 36 farms (FAO, 2023a). The farming system is identical to the one of the Frisa goat, and by the same way, it is a double-purpose goat, with the production of milk, entirely transformed in cheese, and meat based on the slaughter of 38 days old kids (Crepaldi et al., 2002). Information about milk production is quite dated and relatively reliable, but it has been reported a milk yield of 198 L with 2.98% of fat and 2.72% of protein (ARAL (Associazione Regionale Allevatori Lombardia), n.d.). It's a medium-large size goat with a strong constitution (ARAL (Associazione Regionale Allevatori Lombardia), n.d.). Being that this breed hasn't undergone a selection for the phenotypic characteristics, morphologically it shows a wide variety of coat colors, hair length and horn types (Chiatti et al., 2005).

Fig. 14 Lariana goat during winter indoor housing lactating its kid, Germasino (CO), Italy.

Orobica or Valgerola goat origin is unknown, even if some hypothesis about a Southern Italy provenance (supported also by oral tradition of old local breeders) have been formulated (Ajmone-Marsan et al., 2001; Crepaldi et al., 2001). However, a strong genetic identity compared to the others alpine breeds have been demonstrated by different genetic variability's studies (Aimone-Marsan et al., 1999; Crepaldi et al., 2001; Nicoloso et al., 2015). Nowadays this breed can be found on the Orobic Prealps in small farms (10-15 heads per farm on average) mainly in the Sondrio, Como, Lecco, and Bergamo provinces and in particular in Val Gerola, Valsassina, Upper Eastern Lario and Val Brembana (ARAL (Associazione Regionale Allevatori Lombardia), n.d.). The Genealogic Book was activated in 1992, and in 2022 the registered population was 3604 heads in 104 farms (FAO, 2023a).

The farming system is the traditional one (Nicoloso et al., 2015). It's a double-purpose breed, with an average milk yield of 329 L with 4.01% fat and 3.11% protein content (AIA (Associazione Italiana Allevatori), 2020) and meat production of 12-14 kg kids (ARAL (Associazione Regionale Allevatori Lombardia), n.d.). The milk is mainly utilized for the production of Valtellina's Bitto and Storico Ribelle cheeses (both with at most 10% of goat milk), and Orobica goat cheeses (100% Orobica goat milk) (Associazione Formaggi Principi delle Orobie, 2015). It is the only one of the Lombardy breeds with a specific procedural guideline for the production of dairy products of the slow food Orobica goat presidium. Morphologically it's a medium size goat well proportionate and adapted to the mountain pasture with an average weight of 80 kg for the males and 65 kg for the females (Chiatti et al., 2007). The animal is characterized by the presence in both sexes of long horns with a slight helical twist, and by four long hair coats which differ for color and pigment distribution (Farinel, Marin, Nigru and Camosch) (Chiatti et al., 2007). These are the characteristics that allow the differentiation from the other alpine goat breeds, and that testify the probable different origin of Orobica (Crepaldi et al., 2001).

Fig. 15 Orobica goats during the alpine pasture at Preda Rossa, Val Masino (SO), Italy.

Verzaschese or Nera di Verzasca goat is a breed originally coming from Ticino Canton in Switzerland, native to the Verzasca valley, at the border with Italy. During the '70, different of those animals were imported into the peninsula and have been used to replace the very heterogeneous local goat population (G. Gandini et al., 2017). To date, it is farmed mainly in Varese (Val Veddasca), Como (Upper Lario), and Sondrio (Val Chiavenna and Lower Valtellina) provinces (ARAL (Associazione Regionale Allevatori Lombardia), n.d.). Genetic analyses revealed a close relationship with the Di Livo breed, which is bred in valleys adjacent to Verzasca in Italy (Ajmone-Marsan et al., 2001; Crepaldi et al., 2001). The anagraphic register was activated in 2001, and in 2022 the registered population was 779 heads in 9 farms (FAO, 2023a). It is farmed according to a traditional semi-extensive system, and, being a double-purpose breed with a really robust structure, it has a good kid meat production. However, in some areas, such as in the Varese province, it is also milked for the production of typical cheeses (e.g., Formaggella del Luinese (Consorzio per la Tutela della Formaggella del Luinese, 2023)). The average milk yield is 345 L with 3.72% of fat and 3.25% of protein (AIA (Associazione Italiana Allevatori), 2020). It is a large-size goat, with a typical eumelanic black coat with short fine hair, thicker during the cold season. The horns are ibex type (ARAL (Associazione Regionale Allevatori Lombardia), n.d.).

Fig. 16 Verzasca goat during the alpine pasture in Val Bregaglia (SO), Italy.

1.4 PECULIAR PHYSIOLOGY OF THE GOAT

In the past, in veterinary medicine, the cat was often considered to be a miniature dog. The same fate also befell sheep and goats, which were frequently and for a long time considered to be a miniature bovine. Scientific advancements and the efforts of small ruminant practitioners to explore and understand the anatomo-physiological differences among cattle, goats, and sheep have revealed numerous distinct characteristics unique to each species. However, despite these differences, all three species share a common ancestry within the Bovidae family, sharing certain fundamental similarities.

1.4.1 GASTROINTESTINAL APPARATUS PHYSIOLOGY

Capra hircus belongs to the suborder of ruminants, and, as a member of this group, exhibits the characteristic behaviour of rumination. However, compared to sheep and cattle, it has differences in feeding habits and nutrition, which are associated with aspects related to the digestive function and nutrient requirement.

Prof. Dr. R. R. Hofmann was certainly one of the leading researchers in the comparative morpho-physiology of ruminants. With his theory of 'feeding types', he delineated 3 different categories of animals based on their feeding habits, to which correspond a series of anatomo-physiological characteristics. These categories were the 'grass and roughage eaters', such as cattle, sheep, mouflon, the 'concentrate selectors', such as roe deer or moose, and the 'intermediate', i.e. ruminants that morpho-physiologically lie in an intermediate position between the two previous categories, such as goat or deer (Hofmann, 1989a). Hofmann's investigations are still being discussed. To date, it is believed that many of the morpho-physiological differences between ruminants must be ascribed to species-specific characteristics, rather than to the type of ingested forages. However, the most recent classification of ruminants includes two categories, and is a revision of Hofmann's theory. Ruminants can be distinguished on the basis of rumen contents stratification, i.e. the physiology of the forestomaches, in: "cattle-type" ruminants, which are characterized by a high fluid throughput and stratified ruminal contents, and their natural diet is generally based on grass and/or grass and browse mixes; "moose-type" ruminants, which, on the other hand, have low fluid throughput and homogenous, unstratified ruminal contents, and their diet generally consists of high percentages of browse (Clauss et al., 2010). As regards the goat, it was previously classified as an "intermediate" ruminant due to its diet which is markedly characterized by a greater percentage of browse, although, if necessary, it also adapts very well to grass (Devendra, 1989). Indeed, the bipedal position is often assumed by the goat in order to reach even leaves and buds in higher positions, whereas it is absent in species such as sheep (Pfister et al., 1988).

Fig. 17 Goats in bipedal position during free grazing, Sormano (CO), Italy.

However, with the new classification of Clauss et al., goat is included in the "cattle-type" ruminants. This is due to the fact that goats present a stratified ruminal content, in which it is also possible to distinguish the presence of a dorsal gas dome, a non-homogeneous intraruminal papillation pattern with a dorsal area of no or very reduced papillae, and the ability to discern at the level of the reticulo-omasal ostium in the passage/retention between fluid and fibrous particles (Clauss et al., 2022).
Thus, aware of these considerations regarding the general feeding habits and digestive physiology of the goat, we will take in consideration the peculiar morpho-physiological aspects of the gastrointestinal tract of the *Capra hircus*, starting from the mouth till the rectus.

As regards the buccal apparatus, the goat has a narrow opening with a very mobile upper lip which allows it to grasp and select the vegetal essences very refinedly, being able to feed on buds and leaves. Also, the dentition and mandibular and maxilla bones are shaped to favor this behavior. Furthermore, the sense of taste is highly developed in goats compared to sheep or cattle (Hofmann, 1989b). They can distinguish flavors better and have a greater tolerance of bitter, which allows them to feed on a wide variety of plants (Goatcher & Church, 1970). In particular, goats show a physiological adaptation to the ingestion of tannin-rich plant essences, which are normally avoided by other herbivores, also for their bitter taste (Muir, 2011). Tannins are secondary metabolites of plants that have the ecological function of protecting the plant itself from attack by pathogens and herbivores. Against ruminants, they take on anti-nutritive effects, due to their high ability to create strong complexes with proteins, which consequently are made indigestible by the animal ruminal microbiota, as well as reducing the palatability of the feed (Lamy et al., 2011). However, low doses of tannins in the diet of ruminants would seem to favor the ruminal by-pass of forage proteins, subsequently allowing their absorption at the intestinal level. At higher levels, however, this effect is detrimental. Furthermore, possible positive effects of tannins on the regulation of gastrointestinal parasites have been reported (Muir, 2011). In the goat, the constitutive presence of tannin-binding salivary proteins in the saliva allows this species to feed on vegetables rich in tannins without experiencing its negative effects at medium-low doses (Schmitt et al., 2020). In addition, the goat also demonstrates a particularly superior ability than other ruminants to metabolize xenobiotics introduced with the varied plant diet it feeds on. One reason for this is probably the higher hepatic activity of certain enzymes such as cytochrome P450 (Wisniewski et al., 1987). Goat liver has also been studied with regard to the urea cycle. In the last century, hypotheses were formulated regarding a higher efficiency of the urea cycle in goats compared to other domestic ruminants such as cattle (Harmeyer & Martens, 1980). Today, however, there is still very little knowledge on the subject, and more research should be done. The same goes for the rumen forestomach physiology, starting from the ruminal retention time, for which conflicting results are also found in the literature, although recent studies seem to confirm that goats have similar passage rates to other ruminants (Moyo & Nsahlai, 2018). What is certain, however, is that the goat, like the sheep, has very good water recycling, better than the bovine. This can be deduced from the type of faeces produced by sheep and goats. In fact, an important reabsorption of water takes place in the colon, which, together with the peristaltic processes occurring in the last tract of the gastroenteric, determine the typical goat faeces pellets (Magondu et al., 2022). Moreover, in specific local breeds adapted to harsh conditions, such as the Black Bedouin goat, the water balance is extremely controlled in order to allow these animals to survive with a loss of 40% of body weight due to dehydration (Silanikove, 1994). In addition, also the tolerance to salt seems to be higher in goats than in sheep and cattle, and also in this case, some specific local breeds, as reported by FAO, show particular adaptation in that way (FAO, 2015; Giger-Reverdin et al., 2020). Another subject of study that is currently under great development concerns the gastrointestinal and especially the ruminal microbiota of the goat. Compared to other species, investigations are still fragmentary, but what has already been found in other ruminants, such as the strong influence of diet and the major taxa, are confirmed, although the specific functions of each component of the microbiota remain to be defined (Giger-Reverdin et al., 2020).

In conclusion, the goat is an animal that can adapt to extreme climates and environments, maintaining good meat and milk yields even when feeding on poor quality feed rich in toxins or anti-nutritional molecules. Many aspects of the physiology of the digestive system are still poorly investigated in comparison to its sheep and cattle cousins, but in recent years, also in view of the expanding market for goat dairy products in Europe, America, and Asia, research in this regard is increasing.

1.4.2 REPRODUCTIVE APPARATUS PHYSIOLOGY

Small ruminants, unlike other animals with a more recent domestication history, have remained tied to the reproductive physiology of their ancestors, demonstrating even today, with variability, a marked reproductive seasonality. Indeed, these animals are characterized by spontaneous ovulation and are classified as seasonal polyestrous species, as they present several estrous cycles during the reproductive season. Their reproductive activity usually occurs only in a specific period of the year, while, in the remaining part of it, it is absent, especially in females. During this period the animal is in anestrus. As for small ruminants, they are short-day breeders because their breeding season is triggered by negative photoperiod, which occurs when hours of darkness increase and hours of light decrease (e.g., in the North hemisphere, from the summer solstice, 21st June, until the winter solstice, December 21st) (Fatet et al., 2011). However, different factors affect the duration and the onset of the breeding season, mainly latitude (i.e., photoperiod), but also breed, feed availability, climate, presence of male, and breeding system (Fatet et al., 2011).

But how can the main factor affecting seasonal reproduction, i.e., photoperiod, directly influence reproductive activity at the neuroendocrine level?
The resumption of the reproductive activity is determined by the activation of the hypothalamic-pituitary-gonadal axis, which is suppressed during the anoestrus season. The activation of this axis is mediated by a hormone called melatonin, through the retino-hypothalamic pathway (i.e., phototransduction) (Pandi-Perumal et al., 2006). Indeed, this molecule is synthesised by the central nervous system during the hours of darkness, i.e. when there is no blue light reaching the retina. Specifically, when the photoreceptor cells of the retina are not reached by blue light, a nerve impulse is transmitted from the retina to the suprachiasmatic nuclei of the hypothalamus, then to the superior cervical ganglion via preganglionic sympathetic fibres, and from there to the pineal gland or epiphysis. This gland is responsible for the synthesis and secretion into the bloodstream of melatonin. Melatonin has amphiphilic properties that give it the ability to diffuse easily across cell membranes, in particular, is able to diffuse across the blood-brain barrier (Zhao et al., 2019). The melatonin secretion varies along the year, allowing for an adaptive response to changes in the environment (Todini et al., 2011). Therefore, in small ruminants, during the reproductive season, longer peaks of melatonin will occur, as the number of hours of darkness that trigger melatonin synthesis increase during this season (Revel et al., 2009). However, the mechanism through which melatonin activates the hypothalamic-pituitary-gonadal axis, is still under investigation. Scientific research has, by the way, demonstrated that through some mediators, melatonin is able to induce an increased pulsatile secretion of gonadotropin-releasing hormone (GnRH) by the hypothalamus. The increased pulsatile release of GnRH is then responsible for the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by the pituitary gland. These hormones then reach the gonads and induce the resumption of the gonadal activity (Revel et al., 2009). The synthesis and secretion of melatonin is regulated at several levels, and in particular, the activity of the enzymes involved in its synthetic pathway is closely linked to the light impulses that reach the retina (Martínez-águila et al., 2021). To date, melatonin has been demonstrated to have diverse physiological actions. Its effects depend on the location and types of melatonin receptors. Specifically, two high-affinity G-protein-coupled receptors called MT1 and MT2 have been identified to be responsible for regulating several cellular and physiological processes, among which the reproductive activity. Both receptors are expressed by a wide range of tissues. Moreover, melatonin also acts through mechanisms not mediated by receptors, for example, acting as an antioxidant molecule (Pandi-Perumal et al., 2006). Pharmacologically and through light regulation mechanisms, the effects of melatonin on the hypothalamic-pituitary-gonadal axis can be manipulated in order to anticipate the reproductive season and induce cyclicity in small ruminants (Chemineau et al., 1992), as well as to advance the attainment of puberty (Pool et al., 2020). Finally, research in recent years has investigated other non-reproductive side effects of melatonin. A surprisingly wide range of other effects on the organism have been demonstrated, and pharmacological treatment with synthetic melatonin has often proved useful in regulating them. Among these effects are the antioxidant (Hacışevki & Baba, 2018), seasonal moulting (Galbraith, 2010; Santiago-Moreno et al., 2004), temperature and cardiovascular regulator (Duan et al., 2019), immunomodulator (Contreras-Correa et al., 2023a), microbiota-regulator (Contreras-Correa et al., 2023b), and regulator of skeletal muscle growth and development, with the important consequences it may have on the productive performance of small ruminants (Ma et al., 2022).

Thus, as previously mentioned, small ruminants are seasonal polyoestrous species, and the term polyoestrous refers to the fact that they have several successive estrous cycles during the breeding season. The average duration of the estrous cycle in goats is 21 days, with variability related to age, the period of the breeding season, among other factors (Evans, 2003). Although the physiology of the goat's estrous cycle is in many aspects superimposable on that of the sheep or cow, some peculiarities of the former should be noted. For instance, the number of follicular development's waves that occur during the estrous cycle in the goat can vary from 2 to 6, although on average there are 3-4 (Evans, 2003). The last wave generally leads to ovulation of more dominant follicles, usually 2 (Evans, 2003). During the estrous period, the goat displays estrous behaviour, which on average lasts 36 hours (Fatet et al., 2011). The male also displays a sequence of sexual behaviour during the breeding season that is very characteristic of the species (Sánchez Dávila & Pérez Muñoz, 2021).

Fig. 18 Majorera buck in self-marking attitude, Arucas, Gran Canaria, Spain.

The duration of gestation, on the other hand, is similar between sheep and goats, with an average of 150 days, subject to numerous variations depending on age, number and sex of foetuses, breed, time of year (Fatet et al., 2011). During this period, the main peculiarity that allows a clear distinction between the gestation physiology of the two small ruminants is the production of progesterone. Indeed, in the goat, progesterone, which enables pregnancy to be maintained, is produced only by the *corpus luteum*(s) present in the ovary, during the entire duration of the gestation (Sheldrick et al., 1981), while in the sheep, starting from the first trimester, the production is also supported by the placenta (Baril et al., 1993). This particularity plays an important role when it comes to pharmacological treatments aimed at inducing abortion. Finally, after parturition, one of the most important behavioural differences between sheep and goats is represented by the type of mother-young relationship, being the *Capra hircus* a hider species. Indeed, during the first two weeks, the goat kid and its mother spend a relatively low amount of time together, only during the nursing bouts (Lickliter, 1987).

In conclusion, it is evident that some aspects of the reproductive physiology are similar between the two small ruminants, especially with regard to the seasonality of reproduction. In this area too, research to improve the economic aspects of meat and especially dairy goat breeding has led to broad knowledge on this topic. In fact, a full understanding of the mechanisms underlying reproductive seasonality is fundamental to allow a pharmacological or management approach in breeding, aimed at obtaining a constant production throughout the year of both of milk and kids for meat. However, many aspects still remain to be investigated, as several physiological mechanisms are not yet well understood.

1.4.3 LACTATION PHYSIOLOGY

The first attempt at goat domestication by humans was aimed at ensuring a steady supply of meat for sedentary populations during the Holocene. Only later did the new agro-pastoral societies begin to use *Capra hircus* also for milk production. It is precisely man's consumption of dairy products that have produced one of the most important evolutions in the human kind, with the spread of alleles that codify for the adult persistent lactase mainly in the milk consuming population of northern Europe and Africa (Segurel et al., 2020). Since the selection of specific goat populations for milk production began, the morpho-physiology of the mammary gland of the ancestor of the goat (*Capra aegagrus*) has undergone important changes.

Morphologically, the mammary gland of the goat presents a very important peculiarity at the macroscopic level, on which differences also depend at the physiological level compared to other dairy ruminants. Specifically, the cisternal milk fraction is considerably greater in dairy goats (till 82%) (Torres et al., 2013) than in cows (30%) (Ayadi et al., 2004). This means that milk tends to accumulate inside the cistern between one milking and the next, and that when it is ejected, it is immediately ready to be secreted outside the gland. Hence, there are several repercussions that this aspect has on the milking technique. Compared to the cattle, in the goat the stimulation of the neuro-endocrine reflex for the release of oxytocin does not play such an important role in guaranteeing milk ejection. This is why teat pre-stimulation is a phase that in the milking routine in goat flocks is normally not carried out, or in any case coincides with the milking cluster attachment (Dzidic et al., 2019). However, a bimodal milk ejection curve can sometimes be observed in goats, where the first peak is due to the release of cisternal milk and the second of alveolar milk due to myoepithelial cells contraction after oxytocin release (Šlyžienė et al., 2020). These animals produce more milk with higher total solids content, being that the alveolar milk can contain up to 75% of the total milk fat (Labussière, 1988).

However, if this represents the main directly appreciable morpho-physiological mammary gland's peculiarity of goats, at the level of the secretory parenchyma there is another one with equally important repercussions on milk quality. In fact, milk secretion at the level of the alveolar epithelium is apocrine in the goat, while in the cow it is merocrine (Wooding et al., 1970). This results in the release of cytoplasmic particles into the milk, which in a low percentage of cases may also contain nuclear fragments (Dulin et al., 1982). This is responsible for a significantly higher somatic cell count (SCC) in goat milk than in cow milk, which also has a very different compositional profile. In fact, in goat milk, the SCCs are in a greater proportion of polymorphonuclear neutrophilic leukocytes, followed by macrophages and lymphocytes. Finally, the epithelial cells represent just a small portion of goat milk (Dulin et al., 1982). Knowledge of this particularity is crucial when assessing SCC, as only devices using DNA-specific procedures should be utilized to not also count cytoplasm particles. Furthermore, it must also be taken into account that factors causing a significant rise in SCC are an increase in DIM (days in milk) due to the effect of reduced milk production and somatic cells concentration, parity, and any event causing physiological stress (Paape et al., 2001).

From a composition point of view, goat milk differs in several aspects from cow's one, which is why it is often recommended for people with allergy problems or poor cow's milk digestion (Alkaisy et al., 2023). In fact, as far as the fat component is concerned, the fat globules in goat milk are on average smaller in size than in cattle milk, which makes this food more digestible, being more easily attacked by lipases (Park et al., 2007). In addition, the fatty acid profile of goat milk also shows peculiarities. First and foremost is the high proportion of short and medium-chain fatty acids: caproic acid (C6:0), caprylic acid (C8:0), and capric acid (C10:0). Their presence characterizes goat milk products organoleptically, and is due to the particular function of the enzyme fatty acid synthase, which is able to interrupt the synthesis earlier than, for example, in the cow, leading to a shortening of the carbon chain (Zhu & Luo, 2017). Regarding the protein fraction, on the other hand, the concentration variability of α_{S1} casein is responsible for the hypoallergenic reputation of goat's milk. Indeed, this protein seems to be related to cow milk allergy, and in goats its expression, which can vary from null to strong, depends on the genetic polymorphisms of this casein protein (Ballabio et al., 2011).

Finally, the lactation in dairy goats usually lasts between 200 and 305 days, even if recent models of extended lactation are being used in intensive farming, with goats having an inter-pregnancy interval of up to 24 months (Salama et al., 2005). However, in double-purpose goats, the lactation is usually shorter (Suranindya et al., 2020). The lactation curve is often and typically flatter than what is observed in cattle, with a lower peak of lactation. However, milk yield, photoperiod, and parity order are among the main factors that can affect the shape of the curve (Arnal et al., 2018).

In conclusion, from the point of view of the milk product, the peculiarities of the goat are many and of particular interest, especially for its use as a substitute for cow's milk. From a physiological point of view, there are still many cellular and non-cellular mechanisms at the basis of the differences between goat milk and that of other ruminants that still need to be clarified, first and foremost the mode of *de novo* production of short and medium-chain fatty acids. However, given the great interest shown in recent years by consumers for various goat dairy products, investigations into the properties of milk from these animals are increasing.

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2 AIMS

The protection of livestock biodiversity is a goal for the coming decades that numerous national and international organizations, such as the FAO, with the strong support of the scientific community, have recognized and embraced. With regard to the domestic goat species, different investigations all over the world have demonstrated the important and relevant physiological peculiarities resulting from the adaptation to extreme environments of different goat breeds. In Italy, studies in this sense are still rather limited. In particular, with regard to the goat breeds of the Italian Central Alps, such as the Frisa Valtellinese, the Orobica, the Lariana or the Verzaschese, very little is still known about the physiological aspects that characterize them, both from a productive and reproductive as well as a metabolic and immunological point of view.

For these reasons, the aim of this project is to characterize, from a physiological point of view, some of the local goat breeds in Northern Italy, comparing their productive, reproductive, metabolic, and immunological aspects with those of transboundary goat breeds reared in the same territory.

Specifically:

- Regarding the production, the lactation characteristics of local and transboundary goat breeds under the same semi-extensive farming system were investigated.
- Regarding the reproduction aspects, the polymorphism of the MTNR1A melatonin receptor was investigated in different autochthonous and cosmopolitan goat breeds.
- Regarding the metabolic and immunological aspects, the colostrum quality was investigated in different local and transboundary goat breeds; the baseline hematological parameters were evaluated and compared among autochthonous and cosmopolitan goat breeds under the same semi-extensive farming system; the heavy metals, trace elements concentrations, and cortisol concentrations were evaluated during vertical transhumance in a local goat breed.

3 DESCRIPTION OF THE STUDIES

3.1 LACTATION CHARACTERISTICS IN ALPINE AND NERA DI VERZASCA GOATS IN NORTHERN ITALY: A STATISTICAL BAYESIAN APPROACH

The data shown in this chapter refer to the already published paper: Agradi, S., Gazzonis, A. L., Curone, G., Faustini, M., Draghi, S., Brecchia, G., Vigo, D., Manfredi, M. T., Zanzani, S. A., Pulinas, L., Sulce, M., Munga, A., Castrica, M., & Menchetti, L. (2021). Lactation characteristics in alpine and nera di verzasca goats in northern italy: A statistical bayesian approach. Applied Sciences (Switzerland), 11(16), 7235. https://doi.org/10.3390/app11167235

Introduction

Goat (*Capra hircus*, L.) has been, along with sheep (*Ovis aries*, L.), the first species to be domesticated by humankind about 11,000 years ago (Zheng et al., 2020). During contemporary history, the world goat population has been constantly increasing. In particular, starting from 1990, it has risen from about 600 million to over one billion in 2019 (FAO, 2021). The world production of goat-derived products has shown a positive trend in the last three decades (FAO, 2021). That trend could be attributed to a shift in food preferences in developed countries (Castrica et al., 2020) and to the increase in semi-desertic areas with impossibility to raise grazer animals, rather than browser, in underdeveloped and developing countries (Miller & Lu, 2019). India and China are the countries with the highest number of these animals, followed by different African and Asian smaller nations, and are, respectively, the world's largest producers of goat milk and meat (FAO, 2021). However, it is likely that goat meat and, in particular, milk productions data from FAO are underestimates of real values, being that in many countries there is a traditional local production for home consumption that is not reported in the official statistics.

Differences in chemical composition between cow milk and goat milk have been deeply investigated (Park et al., 2017). Several health benefits (scientifically proved or not) to people associated to goat milk are in likelihood to be one of the reasons for its increased consumption. In particular, it is believed that goat milk has lower allergenic properties when compared to cow milk. For that reason, it is usually used as a substitute of cow milk in children with a milk allergy (Haenlein, 2004). However, it has been pointed out that the hypoallergenic characteristic of goat dairy products depends on the concentration of α_{s1}-casein. That, in turn, depends on the wide variety of genetic polymorphisms of this casein protein (Ballabio et al., 2011).

In Italy, goat milk production has been constantly increasing in recent decades, achieving nearly 450,000 quintals in 2019 (ISTAT, 2021). However, in the peninsula, it represents a lower production among the main dairy domesticated species (cow, sheep and buffalo) (ISTAT, 2021). The diffusion of goat milk consumption, as in the dairy cattle sector, led to a flattening in the genetic characteristics of cosmopolitan goat breeds, which are mainly used for milk production (Agradi et al., 2020; Sandrucci et al., 2019). In Italy, the knowledge about local goat breeds is sparse and rare in scientific literature (Claps et al., 2014, 2016; Currò, Manuelian, De Marchi, Claps, et al., 2019; Currò, Manuelian, De Marchi, De Palo, et al., 2019; Currò et al., 2020; Manuelian et al., 2020; Tripaldi et al., 1998; Vacca, Stocco, Dettori, Pira, et al., 2018). The few studies that have been conducted were mainly focused on the comparison of dairy products' quali-quantitative characteristics in different Mediterranean autochthonous goat breeds versus Saanen, Alpine and Maltese goats. The studies have highlighted a higher milk yield of the cosmopolitan breeds, but a lower milk nutritional quality (Currò, Manuelian, De Marchi, Claps, et al., 2019; Currò, Manuelian, De Marchi, De Palo, et al., 2019; Currò et al., 2020; Manuelian et al., 2020; Tripaldi et al., 1998; Vacca, Stocco, Dettori, Pira, et al., 2018). Additionally, the coagulative aptitude and nutritional value of cheeses were higher in the Mediterranean breeds (Currò et al., 2020; Vacca, Stocco, Dettori, Pira, et al., 2018; Vacca, Stocco, Dettori, Summer, et al., 2018), and the colostrum from Garganica goats versus Saanen or Maltese has shown interesting oligosaccharides concentrations (Claps et al., 2014, 2016).

Alpine goat (Camosciata delle Alpi) is a dairy breed. It is originally from the mountainous cantons of Bern, Freiburg, Glarus and Graubünden in Switzerland. However, in recent decades, it has been exported in several other countries such as France, Italy and Germany, but it is also reared outside Europe. In Italy, it is mainly found in the Alpine regions, especially in Piedmont and Trentino-Alto Adige. The numerosity in 2020 was of 67,780 heads in the National Zootechnical Register. This breed is a medium–large size goat, originally sturdy, strong, well-adapted to the climate and mountainous geographical conditions. However, the selection for milk production has led to a weakening of its rusticity characteristics.

The Verzasca (Nera di Verzasca or Verzaschese) is a double aptitude breed. It is originally from the Verzasca Valley, in the canton of Ticino in Southern Switzerland. In Italy, it is mainly reared in the provinces of Como, Varese and Verbano-Cusio-Ossola in Northern Lombardy and Piedmont. The numerosity in 2020 was of 2248 heads in the National Zootechnical Register. It is also a medium–large size goat and is characterized by a high rusticity and resistance both to high and low temperatures. In Italy, it is considered an autochthonous breed of limited distribution among the forty-six for which the national association of pastoralism (AssoNaPa) keeps a herd book.

The aim of the present paper is to assess the differences between the basic lactation variables in Alpine and Verzasca goats from a Bayesian point of observation.

Materials and Methods

Animals

For this study, 71 female goats were selected. They were of Alpine (*n* = 37) and Verzasca (*n* = 34) breed and managed under the same semi-extensive farming system in the mountains near Verbano Lake, in Varese province, Northern Italy. The farm was located at 980 m above sea level (m.s.l.), but goats were kept under indoor housing at that altitude only during winter, when they were fed hay *ad libitum* with an increasing supplementation of concentrate from the dry period to early lactation (from 300 g/day to 600 g/day). From March to November, the goats were free to graze on alpine pasture nearby the housing (200 hectares (ha) between 900 and 1550 m.s.l.). The diet was based on the vegetable essences typical of that area, as described by Maggioni et al. (Maggioni et al., 2004). Birth season was from 11th of January to the 21st of March, and since parturition goats were milked twice a day until September.

Only clinically healthy goats that had eutocic delivery and regular post-partum were included in the study. The mean rectal temperature ranged between 38.5 and 39.7 °C. Mean Body Condition Score (BCS) was 2.8 for Alpine goats, 2.7 for Verzasca ones. Mean age was 4.21 ± 2.27 for Alpine goats and 4.79 ± 2.34 for Verzasca goats.

In order to evaluate the effects of age and days in milk (DIM) on response variables, a subdivision in classes was performed. The age of goats was expressed as age class:

Class 1–1 to 2.9 years
Class 2–3 to 4.9 years
Class 3–5+ years

Similarly, the DIM of goats were subdivided in:

Class 1–0 to 30 DIM
Class 2–31 to 50 DIM
Class 3–51 to 100 DIM
Class 4–101 to 150 DIM
Class 5–150 + DIM

Sample Collection and Analysis

Milk samples were monthly collected from February to September and analyzed by the Milk Standard Laboratory of the Italian Breeder Association (A.I.A.). Individual milk yield was collected at the official Milk Recording (International Committee for Animal Recording – ICAR, 2008). A total of 121 milk collections were performed on Alpine goats, and 170 samplings were performed on

Verzasca subjects. The milk was obtained from hand milking before morning milking. First streams of foremilk were discharged and milk was collected in Corning^{TM} Falcon 15 mL conical centrifuge tubes. The samples were immediately refrigerated and sent to the laboratory in 12 h, where they were analyzed in duplicate.

The distribution of the milk collections in terms of age class and DIM class are reported in Table 1.

Table 1. Sample distributions for breed, age class and days in milk class (DIM class) for the enrolled goats.

Breed	**Age Class (Years)**	***n***
Alpine	1–2.9	45
	3–4.9	21
	5+	55
Verzasca	1–2.9	35
	3–4.9	78
	5+	57
	DIM class (days)	
Alpine	0–30	9
	31–50	20
	51–100	24
	101–150	37
	150+	31
Verzasca	0–30	7
	31–50	27
	51–100	33
	101–150	46
	150+	57

Fat, protein and lactose percentage were determined by infrared method (Laboratorio Standard Latte—Associazione Italiana Allevatori (LSL-AIA) http://www.aia.it/lsl/index.htm, accessed on 5 April 2021). The somatic cell count (SCC) was determined by fluoro-opto-electronic counting (Bentley SOMACOUNT 150, Bentley Instruments, Chaska, MN, USA) and was expressed as log10 SCC.

Moreover, the fat/protein and the fat/lactose ratios were calculated on raw data, since fat/protein and fat/lactose ratios in milk are valuable lipomobilization indexes in cows, as well as indexes of pathologies and post-partum disorders (Paudyal et al., 2016; Toni et al., 2011).

Statistical Data Analysis

On raw data, the univariate descriptive statistics (mean and standard deviation) were calculated. To assess the effects of breed, age and lactation period, a Bayesian approach to ANOVA was adopted. Bayes' ANOVA analysis is essentially based on the evaluation of one or more effects given a prior probability of an outcome. ANOVA Bayesian analysis was performed with the software Jamovi for Windows. The significance (strength) of posterior probabilities was derived from the Jeffreys classification of Bayes Factor (BF10) or, otherwise, the inverse (BF01) (Jarosz & Wiley, 2014). A notation similar to the frequentist statistics results was adopted, following the Jeffreys' suggestions, resumed in Table 2.

Table 2. BF10, inverse BF10 (Jarosz & Wilet, 2014) and arbitrary significance asterisks, inserted by authors.

Evaluation	*BF10*	*Inverse BF10*	*Significance*
Anecdotal	1–3	1–0.33	ns
Substantial	3–10	0.33–0.1	ns
Strong	10–30	0.1–0.03	*
Very strong	30–100	0.03–0.01	**
Decisive	>100	<0.01	***

The different asterisks indicate different levels of statistical significance: strong (*), very strong (**), decisive (***).

The notation with asterisks was chosen arbitrarily by authors considering the strength of evidence.

Data were processed for univariate descriptive analysis: mean value and standard deviation were considered for data summarization. The effects of breed, age class and days in milk class on response variables were analyzed by the Bayesian ANOVA analysis procedure (Jamovi ver. 1.1.7.0. for Windows). The model analyzed was resumed in the following relation:

$$\textit{Output variable}_{ijkl} = \mu + \textit{breed}_i + \textit{age}_j + \textit{DIM}_k + \varepsilon_{ijkl}$$

where:

- μ = grand mean
- *breed* = fixed factor for breed
- *age* = fixed factor for age class
- *DIM* = fixed factor for days in milk class
- ε = error

The breeds differences on age class and DIM classes were based on the interaction between factors.

Moreover, the Bayesian correlation coefficients between variables in each breed were calculated.

Results

The summary descriptive statistics expressed as mean ± standard deviation are reported in Table 3.

Table 3. Descriptive statistics for milk parameters in Alpine and Nera Verzasca goats.

	Breed	**Milk Yield (L/d)**	**Fat (%)**	**Protein (%)**	**Lactose (%)**	**SCC [1] (10^3/mL)**
Mean ± SD [2]	Alpine	2.14 ± 1.03	3.18 ± 0.78	3.15 ± 0.39	4.43 ± 0.28	1216 ± 2828
	Verzasca	1.68 ± 0.84	3.27 ± 0.82	3.24 ± 0.41	4.55 ± 0.26	798 ± 1734
Min − Max	Alpine	0.10 − 5.20	1.23 − 4.62	2.27 − 4.46	3.62 − 5.05	48 − 27987
	Verzasca	0.10 − 4.00	1.52 − 4.78	2.48 − 4.77	3.66 − 5.14	27 − 17311

[1] SCC—somatic cell count; [2] SD—standard deviation.

The effects of breed, age class and DIM class on milk yield are reported in Table 4. The Bayesian analysis put in evidence a significant effect of breed and DIM: Alpine goats yielded a higher amounts of milk in a single milking than Nera Verzasca, when age class or DIM class were considered (Table 4 and Figure 1a,b). In both breeds, daily milk yield reached a zenith in the period 51–100 DIM, more evident in Alpine goats than Verzasca ones. Then, the milk yield progressively decreased, reaching values significantly lower than the ones at the beginning of lactation. Although milk fat and protein percentages did not differ between breeds, a decreasing trend in fat and protein percentage was observable during aging (statistically significant only for protein percentage) (Table 4 and Figure

1c,d); both the milk fat percentage and milk protein percentage had shown a “U” shape trend when considering DIM classes (Figure 1e,f). These two parameters, in fact, assumed their higher values at the beginning and at the end of lactation; while in the middle of it, they reached the lowest values. Milk lactose percentage was significantly higher in the Nera Verzasca than in the Alpine breed. This difference was maintained during all the lactation periods considered and maintained a constant, decreasing trend (Table 4 and Figure 1g,h). The fat/protein ratio in milk, as a fat mobilization index, changed significantly with DIM (Figure 1j), without evidencing differences between breeds or age class (Figure 1i). The fat/lactose ratio as a secondary fat mobilization index statistically overlapped to the fat/protein ratio, with significant influence of DIM class (Table 4 and Figure 1l). Somatic cell count (SCC) significantly increased both with age and DIM class, but no statistically significant differences were shown between breeds, although a higher value in SCC was observed in the Alpine breed milk (Table 3 and Table 4 and Figure 1m,n).

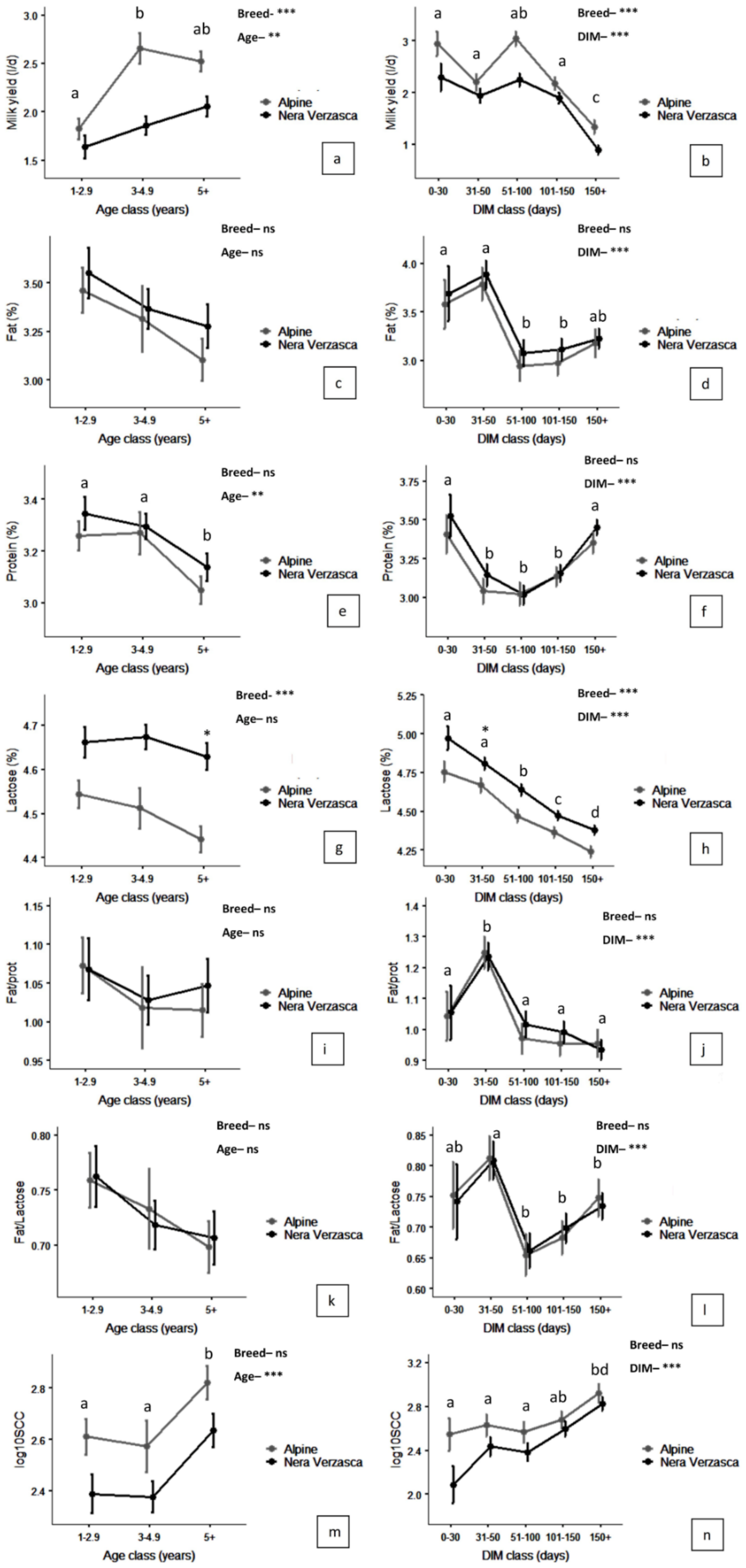

Figure 1. Patterns of milk variables in Alpine and Nera di Verzasca goat breeds for age and days in milk (DIM), respectively. The effects of breed, age and DIM have been evaluated on milk yield (**a**,**b**), milk fat % (**c**,**d**), milk protein % (**e**,**f**), milk lactose % (**g**,**h**), milk fat/protein ratio (**i**,**j**), milk fat/lactose ratio (**k**,**l**) and on somatic cell count (**m**,**n**). Letters indicate a significant difference in age or DIM groups. Asterisks in the graphs indicate

the age/DIM period with significant differences between breeds. Data were resumed as mean values and standard error of mean.

Table 4. Bayesian ANOVA results for milk variables. Significance symbols were derived from the BF10 values as reported in the text. The different asterisks indicate different levels of statistical significance: very strong (**), decisive (***).

Milk Yield (L/Day)				
Models	P(M)	P(M|data)	BF10	Signif
Breed	0.0769	3.53 x 10^{-30}	503.38	***
Age class	0.0769	3.88 x 10^{-32}	5.54	**
DIM[2] class	0.0769	2.01 x 10^{-8}	2.86 x 10^{24}	***
Fat (%)				
Breed	0.0769	5.29 x 10^{-8}	0.19	Ns
Age class	0.0769	4.53 x 10^{-7}	1.64	Ns
DIM[2] class	0.0769	0.31	1.11 x 10^{6}	***
Protein (%)				
Breed	0.0769	2.34E x 10^{-11}	0.66	Ns
Age class	0.0769	3.22 x 10^{-9}	90.83	**
DIM[2] class	0.0769	0.01	4.37 x 10^{7}	***
Lactose (%)				
Breed	0.0769	4.31 x 10^{-31}	170.17	***
Age class	0.0769	4.19 x 10^{-33}	1.65	Ns
DIM[2] class	0.0769	1.24 x 10^{-7}	4.91 x 10^{25}	***
Fat/Protein				
Breed	0.0769	6.75 x 10^{-10}	0.13	Ns
Age class	0.0769	4.52 x 10^{-10}	0.09	Ns
DIM[2] class	0.0769	0.81	1.56 x 10^{8}	***
Fat/Lactose				
Breed	0.0769	9.09 x 10^{-5}	0.13	Ns
Age class	0.0769	3.39 x 10^{-4}	0.49	Ns
DIM[2] class	0.0769	0.55	785.59	***
$\log_{10}$SCC [1]				
Breed	0.0769	3.63 x 10^{-9}	2.85	Ns
Age class	0.0769	9.47 x 10^{-7}	744.22	***
DIM[2] class	0.0769	1.20 x 10^{-4}	94,248.97	***

[1] SCC—somatic cell count; [2] SD—standard deviation.

Table 5 and Table 6 show the Bayesian correlation coefficients between milk variables in the two examined breeds. Correlations appeared similar between breeds, except for milk yield that differently correlated with some variables in different fashions if the Alpine or Nera Verzasca breed was considered. Milk fat percentage was strongly negatively correlated with milk yield, but this correlation was stronger in the Alpine than in the Nera Verzasca breed (Table 5). Analogue conclusions can be drawn for the fat/protein ratio (Table 5). An opposite behavior could be seen considering the milk yield and protein percentage (Table 5), with a stronger negative correlation in the Nera Verzasca breed with respect to the Alpine goat.

Table 5. Bayesian correlation coefficients for milk variables in Alpine goat breed. Asterisks evidence the significance of BF$_{10}$ as reported in the text.

	BF$_{10}$	**Milk Yield**	**Fat**	**Protein**	**Lactose**	**F/P [2] Ratio**	**F/L [3] Ratio**	**$\log_{10}$SCC [1]**
Milk Yield	r	—						

	BF$_{10}$	—						
Fat	r	−0.41	—					
	BF$_{10}$	***	—					
Protein	r	−0.31	0.29	—				
	BF$_{10}$	**	*	—				
Lactose	r	0.17	0.41	0.02	—			
	BF$_{10}$	*	***	*	—			
F/P [2] Ratio	r	−0.28	0.88	−0.18	0.38	—		
	BF$_{10}$	*	***	ns	***	—		
F/L [3] Ratio	r	−0.49	0.97	0.31	0.17	0.85	—	
	BF$_{10}$	***	***	**	ns	***	—	
log$_{10}$SCC [1]	r	−0.41	0.21	0.27	−0.49	0.12	0.3	—
	BF$_{10}$	***	ns	ns	***	ns	***	—

[1] SCC—somatic cell count; [2] F/P ratio—fat to protein ratio; [3] F/L ratio—fat to lactose ratio. The different asterisks indicate different levels of statistical significance: strong (*), very strong (**), decisive (***).

Table 6. Bayesian correlation coefficients for milk variables in Nera di Verzasca goat breed. Asterisks evidence the significance of BF$_{10}$ as reported in the text.

	BF$_{10}$	**Milk Yield**	**Fat**	**Protein**	**Lactose**	**F/P [2] Ratio**	**F/L [3] Ratio**	**log$_{10}$SCC [1]**
Milk Yield	r	—						
	BF$_{10}$	—						
Fat	r	−0.34	—					
	BF$_{10}$	***	—					
Protein	r	−0.42	0.33	—				
	BF$_{10}$	***	***	—				
Lactose	r	0.24	0.39	0.07	—			
	BF$_{10}$	*	***	Ns	—			
F/P [2] Ratio	r	−0.16	0.88	−0.15	0.37	—		
	BF$_{10}$	*	***	Ns	***	—		
F/L [3] Ratio	r	−0.42	0.97	0.34	0.17	0.85	—	
	BF$_{10}$	***	***	***	Ns	***	—	
log$_{10}$SCC [1]	r	−0.43	0.16	0.23	−0.32	0.07	0.25	—
	BF$_{10}$	***	*	ns	***	*	*	—

[1] SCC—somatic cell count; [2] F/P ratio—fat to protein ratio; [3] F/L ratio—fat to lactose ratio. The different asterisks indicate different levels of statistical significance: strong (*), decisive (***).

Discussion

Goat milk components are subject, as in other species, to a plethora of influencing factors, either biological or management factors, as evidenced by Sandrucci et al. (Sandrucci et al., 2019). It is, therefore, difficult to isolate these aspects in order to have a complete picture of the multiple interactions between factors.

The present paper evidenced and compared the characteristics of lactation in a local Italian/Southern Switzerland goat breed (Nera Verzasca) and a cosmopolite breed as the Alpine goat, both reared in the same farm; thus, with the same management conditions. These results can help to characterize and to protect this rare, endangered, local goat breed.

Currò et al. report, on research regarding local goat breeds, a significant influence of DIM on lactation traits (Currò, Manuelian, De Marchi, De Palo, et al., 2019); the local breeds' milk yield was lower than cosmopolite breeds', but accounted for a reduced number of SCC and higher lactose.

These features overlap with our experimental data: Nera Verzasca goats had a reduced milk yield with respect to the Alpine breed, but the SCC showed a tendency to be lower in the local Nera Verzasca breed. It must be highlighted that milk protein concentration was similar between breeds; this feature can be advantageous for Nera Verzasca goats when milk is considered for cheesemaking.

The average daily milk yield in our Alpine subjects appeared similar to the values reported for Alpine goats by Zeng et al. (Zeng et al., 1997) (2.14 kg/day vs. 1.98 kg/day in our data and Zeng et al., respectively). However, this study was carried out in 1995 and just on primiparous goats. Another study conducted in Italy in 2018 which considered a different type of farming system, including an intensive one, recorded a daily mean yield of 2.57 kg for the Alpine goat (Vacca, Stocco, Dettori, Pira, et al., 2018). That higher value could be explained both by different farm management systems (mainly referring to the diet) and the more than 20 years of progress made in the artificial selection for production traits. Similar values were also obtained in recent studies conducted in Croatia on Alpine goats (Piliena & Jonkus, 2012; Špehar et al., 2019). On the other side, Verzasca goats showed an average milk yield of 1.68 kg/day in our study. That is an expected result, as stated above, because that breed was not strictly selected as in the case of the Alpine goat for milk production, but was actually a double aptitude breed. The effect of parity (i.e., age) shown in our study was in agreement with other previous investigations (Crepaldi et al., 1999; Piliena & Jonkus, 2012; Salvador & Martinez, 2007; Zeng et al., 1997) which highlighted a common trend: the first three lactations are characterized by a lower milk production compared to the 4th and 5th lactations, and are followed then by a decrease in the milk yield. This trend is the result of an increasing udder volume through lactations given by the progressive sum of new and old secretory parenchyma at every parturition. However, achieving the age of 5 years old, the organism probably has a reduction in performance. In our study, goats, considering the DIM class, reached a production peak during 51–100 days in milk after a slight depression during the period of 31–50 days of lactation. The same pattern, but with a reduced yield, was followed by the Nera Verzasca breed. This trend through lactation was perfectly confident with what was already present in the scientific literature (Zeng et al., 1997; Zeng & Escobar, 1996), with a progressive increase in the milk yield starting from parturition till achieving a peak about 1 to 3 months post-partum. Then, a gradual decreasing pattern was shown until dry off.

Fat percentage was 3.18% in Alpine and 3.27% in Verzasca goats, not showing a statistically significant difference among breeds. The trend through lactation, with maximum values at the beginning and at the end of it, was likely due to a dilution effect given that caused both the fat and protein percentage to decrease during the higher lactational productive period (Antunac et al., 2001; Piliena & Jonkus, 2012; Zeng et al., 1997).

Even the protein percentage showed no differences between breeds and the same trend through lactation as the fat percentage. However, a significant age effect was also highlighted. The decreasing protein percentage with the increase in age was in agreement with the study of Antunac et al. (Antunac et al., 2001).

The lactose percentage was the only parameter along with milk yield that showed a statistically significant difference between breeds. In particular, Verzasca goats showed a significantly higher value than the Alpine ones. Additionally, lactose showed a decreasing pattern starting from 30 to 50 days post-partum. That result has already been described in the literature (Antunac et al., 2001; Zeng et al., 1997).

SCC was lower but not in a significant way in Verzasca goats (798 × 103/mL) than in the Alpine ones (1216 × 103/mL). In goats it is well documented that increasing the DIM was among the most important factors contributing to an increased cell count in the absence of intramammary infection (Paape et al., 2001; Tedde et al., 2019). In the present experiment, SCC exhibited a constant increase during DIM; moreover, the SCC significantly rose with the subject's age. Although SCC difference was not significant and the patterns appeared similar for both the considered breeds, the Nera Verzasca goat had a lower SCC than the Alpine goat when considering the breed, DIM class and age class. These data showed that the Verzasca breed can maintain lower levels of mastitis maker, suggesting a better resistance against the mammary gland pathogen. This breed has already shown interesting traits regarding the resistance against pathogens, especially concerning gastrointestinal parasites (Gazzonis et al., 2021; Zanzani et al., 2020).

The fat/protein ratio was considered a valuable variable to monitor the nutrient balance (Toni et al., 2011), mainly in early postpartum; Heuer et al. and Toni et al. report that the fat/protein ratio is, in cattle, a reliable predictor for several diseases, fertility problems and variation in milk yield (Heuer et al., 1999; Toni et al., 2011). Toni et al. refer that cows with a higher milk fat/protein ratio (over than 1.5) have a higher probability of disease and culling (Toni et al., 2011). Sandrucci et al. evaluated the milk fat/protein ratio also in goats and resulted significantly influenced by the month of kidding, stage of lactation, herd size, parity and milk production volume (Sandrucci et al., 2019). Our data suggest a parallelism of the fat/protein ratio behavior; among examined goats, the Alpine subjects had a fat/protein ratio above 1.5, making difficult an evaluation of the phenomenon, considering that the cutoff was established for the bovine species.

Another interesting parameter to evaluate the energy balance was the fat/lactose ratio in milk which was indicated in cattle as a valid indicator for mastitis and ketosis (Paudyal et al., 2016; Reist et al., 2002). Our data showed an increased ratio for both breeds around the 31–50 days in milk, which was the period associated with the most elevated energy requirement necessary to cope with the elevated milk yield. Another peak was visible at the end of the lactation; this was due to the physiological reduction in lactose associated with the moderate elevation of fat which was physiological at the end of lactation (Fekadu et al., 2005; Reist et al., 2002).

Correlations between milk traits in Alpine goats vary in literature; Zeng et al. report a lower fat/lactose correlation with respect to our results ($r = 0.14$ vs. 0.41, respectively) (Zeng et al., 1997). Protein/lactose correlation is sensibly higher in the data referred by Zeng et al. compared to our results ($r = 0.25$ vs. 0.02, respectively) (Zeng et al., 1997). Additionally, the protein/SCC and protein/milk yield correlations in our samples were sensibly higher than those reported by Zeng et al. (protein/SCC correlation $r = 0.17$ vs. 0.27 and protein/milk yield correlation $r = 0.04$ vs. −0.31 in Zeng et al. data and our samples, respectively) (Zeng et al., 1997). The SCC/milk yield correlation differs between results reported by Zeng et al. and our data ($r = -0.09$ vs. −0.40, respectively) (Zeng et al., 1997). These correlations appeared overall stronger in our data; this could be due to different management methods and different feeding systems from the standing point of each breed. However, also the study by Mioč et al. investigated the same correlation and shows many results in agreement with ours (Piliena & Jonkus, 2012). In particular, the positive correlation between the milk yield and lactose could be due to the osmotic activity of the latter, which is responsible for a higher milk production when present at higher concentrations. Obviously, because of a sort of dilution effect, milk yield was negatively correlated with the fat percentage, protein percentage and SCC.

Conclusions

The Nera di Verzasca goat, a local breed in Northern Italy, had overall lactation characteristics similar to a cosmopolite breed such as Alpine, with the exception of the milk daily yield reduced with respect to Alpine goats. These aspects can be taken into account in further research to study the motivations of these discrepancies and the effects of these features on the milk transformation.

Moreover, these findings are important in the optic of biodiversity conservation; despite their lower production, local breeds could play a key role in the future, thanks to the ability to guarantee, also in marginal areas, products with a high level of quality and safety, along with a plethora of ecosystem services.

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3.2 POLYMORPHISM OF THE MTNR1A MELATONIN RECEPTOR GENE IN GOAT BREEDS OF NORTHERN ITALY

The data shown in this chapter refer to the submitted paper: Agradi, S., Luridiana, S., Mura, M.C., Cosso, G., Vigo, D., Menchetti, L., Brecchia, G., Carcangiu, V., Curone, G. (submitted October 28, 2023). Polymorphism of the MTNR1A Melatonin Receptor Gene in Goat Breeds of Northern Italy. Animals

Introduction

Melatonin is a hormone that was first isolated and characterized from the bovine pineal gland by dermatologist Aaron Lerner in 1958. It is named for its ability to cause frog skin to lighten by the contraction of melanophores and the fact that it is synthesized from a serotonin molecule (Leerner et al., 1958). Melatonin has since been isolated in all major taxonomic groups, including both invertebrate and vertebrate species. Its synthesis occurs in the mitochondria (or chloroplasts and prokaryotes outside the Animalia kingdom) indicating that it is one of the phylogenetically oldest signaling mechanisms, according to the evolutionary theory of endosymbiosis (Zhao et al., 2019). In vertebrates it is primarily secreted at the level of the pineal gland, secondarily at the level of the retina, gastroenteric, skin, platelets, lymphocytes, bone marrow, and probably in other structures as well (Pandi-Perumal et al., 2006). In higher organisms, it plays a key role in regulating circadian rhythms and reproductive seasonality. The latter function is based on the conversion of information from the photic environment indicating the length of day and night and its translation into a chemical signal, namely melatonin itself and its metabolites (Dubocovich et al., 2010; Zhao et al., 2019). In fact, the synthesis pathway of this molecule is regulated at multiple levels by the light signals received by the retina (Schomerus & Korf, 2005). Specifically, pinealocyte secretion of melatonin peaks at night, during the dark hours (Pandi-Perumal et al., 2006).

Melatonin is deeply involved in the regulation of reproductive activity in small ruminants. Indeed, these animals are counted among the short-day breeders because the resumption of their reproductive activity occurs at the same time as the shortening of the photoperiod (Carcangiu et al., 2015). In fact, the increase in hours of darkness corresponds to an increase in melatonin secretion, which through pathways not yet fully elucidated, results in an increase in tonic GnRH secretion at the hypothalamic level (Malpaux et al., 1999; Revel et al., 2009). However, the melatonin effects are multiple and still under investigation, and depend on the types of receptors with which it interacts once released into the bloodstream and on their localization in the organism. In mammals, there are two melatonin receptors, MT1 and MT2, both of which are G protein-coupled receptors. In particular, MT1 has been identified in different tissues, including the *pars tuberalis* of the pituitary gland and hypothalamus (Dubocovich et al., 2010). Studies indicate that this receptor is the main one involved in the seasonal regulation of the reproductive activity (Weaver et al., 1996; Yasuo et al., 2009). MT1 receptor is encoded by the MTNR1A gene (Jockers et al., 2016). The ovine MTNR1A gene polymorphic RFLP sites were first identified and described by Messer et al. in 1997 (Messer et al., 1997). Following this discovery, different studies in sheep have been conducted to investigate the existence of any relationship between MTNR1A gene polymorphism and variation in reproductive performance (Antonopoulou et al., 2023; Arjoune et al., 2023; Calvo et al., 2018; Fathy et al., 2018; Giantsis et al., 2016; He et al., 2019; Kianpoor et al., 2018; Martínez-Royo et al., 2012; Mateescu et al., 2009; Saxena et al., 2015; Yang et al., 2022). However, in goats, the studies on this matter are still limited. The first investigation on goat MTNR1A polymorphism was conducted in 2002, and evidenced the existence of 7 *MnlI* sites, but none showed polymorphism (Migaud et al., 2002). Since this study, others have been conducted on different goat breeds (Abdolahi et al., 2019; Carcangiu, Vacca, et al., 2009; Chu et al., 2007; Korkmaz Agaoglu et al., 2015; Lai et al., 2013), but to date, no data is available about Italian goat breeds other than Sarda goat, which is a local breed typical of Sardinia region.

On the Italian Alps, the latitude and, consequently, the photoperiod, are different from those of Sardinia region. Frisa, Orobica, and Lariana are three native goat breeds from Northern Italy, which have been bred for centuries in the Alps, and are characterized by a pronounced reproductive seasonality with a birth season running from late December to early March. The Camosciata goat, on the other hand, is a cosmopolitan breed, native to the Swiss Alps, which exhibits the same seasonality as the native mentioned above breeds at similar latitudes. These breeds belong to the same breed cluster. Indeed, the spatial and temporal distancing from the domestication center of the goat at the beginning of Holocene epoch caused genetic divergence among the first goat populations, leading to the differentiation of specific breeds within 3 different clusters: the Asian cluster, the European cluster, and the African cluster (Zheng et al., 2020). The local breeds of Italy belong to the European cluster, whereas the greater part of the breeds investigated for MTNR1A gene polymorphism belong to the Asian one. The hypothesis of this study is that the Alpine arch native goat breeds, that have never been investigated, are genetically different from the Asian goat breeds, and may show significant differences in the allele frequencies of the MTNR1A gene, compared to what has been reported in the scientific literature for year-round estrous breeds (Chu et al., 2007). This study aimed to detect any PCR-RFLP polymorphic sites of MTNR1A using *MnlI* and *RsaI* enzymes and investigated the association among the relative allele frequencies and reproductive seasonality in Northern Italy goat breeds.

Materials and Methods

Animals involved and sample collection

In this study, a total of 108 adult female goats belonging to 4 different breeds were included (i.e., Frisa, n=29; Orobica, n=23; Lariana, n=29; Camosciata delle Alpi, n=27). Frisa Valtellinese or Frontalasca goat, Orobica or Val Gerola goat, and Lariana or Di Livo goat are local breeds that have been bred for centuries in different valleys of North Italy, in the Lombardy Alps. Studies on these autochthonous breeds are very limited, and they are facing extinction because of the progressive abandonment of both their farming and the use of mountainous lands where they are native. Camosciata delle Alpi or Alpine goat is a trans-boundary breed native to Switzerland, mainly bred to produce milk. All 4 breeds involved in this study are characterized by a kidding season that typically extends from late December to early March. In Northern Italian human societies, seasonality has historically been associated with the traditional consumption of goat kid meat during the Easter holidays.

The animals involved in the study were randomly selected from 4 different farms located in Lodi province for Camosciata delle Alpi goats, in Sondrio province for Frisa and Orobica goats, and in Como province for Lariana goats. Thus, the farms involved were all located in the Lombardy region, between the 45 and 46 North latitude parallels. The management of the Frisa, Orobica, and Lariana goat farms was the same, based on a traditional semi-extensive farming system for the production of cheese and kid meat. Camosciata delle Alpi goat was bred for the production of both cheese and milk following a semi-intensive farming system. However, all the goats were kept under natural photoperiod since birth. Males were introduced in the flocks from mid-July until early November. Before inclusion in the investigation, all goats were clinically examined, and only healthy animals were enrolled. Only pluriparous goats were included in the study, and mean age (and standard deviation) was, 5.7±2.7, 5.8±1.8, 5.3±1.8, 3.1±1.1 years for Frisa, Orobica, Lariana, and Camosciata delle Alpi goats, respectively. The number of kids born to each goat breed included in the study for the kidding season of the year under investigation, is reported in Table 1.

Table 1. Litter size per breed.

Litter size	Breed			
	Frisa	Orobica	Lariana	Camosciata delle Alpi
1	18 (62.1%)	23 (100.0%)	24 (82.8%)	5 (18.5%)

2	11 (37.9%)	0 (0.0%)	5 (17.2%)	20 (74.1%)
3	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (7.4%)
Total	29	23	29	27

The kidding season occurred between the following timeframes: from December 16th to January 4th for Frisa goats, from February 14th to March 23rd for Orobica goats, from De-cember 21st to January 8th for Lariana goats, and from January 11th to February 17th for Camosciata delle Alpi goats. Ten ml of blood were sampled from each goat by jugular venipuncture using 18G disposable needles into sterile vacuum tubes containing K3EDTA as an anticoagulant (BD Vacutainer Systems, Franklin Lakes, New Jersey, U.S.). Blood samples were then stored at -20°C until analysis. The collection of blood samples was performed with a strict commitment to animal welfare in accordance with the current Italian legislation. The study was conducted with the approval of the Institutional Animal Care and Use Committee of Università degli Studi di Milano (Per-mission OPBA_04_2021).

Genomic DNA preparation and primers sequences

Genomic DNA was extracted from whole blood using a commercial kit (Purgene, Gentra, Minneapolis, Minnesota, U.S.) and stored at -20°C till further analysis.

For the polymerase chain reaction (PCR), 100 ng of genomic DNA were used with primers by Messer et al. (Messer et al., 1997) synthesized by Sigma Genosys Ltd (Pampisford, Cambs, UK). The primers were of the sequence of Reppert et al. (Reppert et al., 1994), which are part of exon II of the MTNR1A ovine gene (GenBank U14109), from position 285-304 (Carcangiu, Vacca, et al., 2009; Chu et al., 2007; Migaud et al., 2002) (sense primer: 5'- TGT GTT TGT GGT GAG CCT GG-3') and 1108-1089 (antisense primer: 5'-ATG GAG AGG GTT TGC GTT TA-3'). Fifty µL of total volume were used to start PCR. Specifically, the starting volume contained: 5 µL of 10× PCR buffer (which was a solution composed of 50 mM L/L KCl, 10 mM Tris-HCl (pH 8.0), 0.1% Triton X-100), 3 µL of 1.5 mM MgCl2, 8 µL of 0.2 mM solution containing each dNTP, 1 µL of 10 pM/L solution containing each primer, 100-150 ng of extracted genomic DNA, and 5 U of Taq DNA polymerase (HotMaster Taq DNA Polymerase, Eppendorf AG, Hamburg, Germany). Then, the PCR was run according to the following protocol: denaturation at 94°C for 5 min, followed by 35 cycles shared in denaturation at 94°C for 1 min; annealing at 62°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 10 min. The cycler used was Mastercycler® Gradient (Eppendorf AG, Hamburg, Germany).

After the PCR, the products were separated by electrophoresis on 2% Agarose gel (GellyPhor, Euroclone, UK). A 100 bp DNA marker was also run with the PCR products (Invitrogen, Carlsbad, CA, USA). Finally, the separated products were digested with 5 U of *MnlI* enzyme (New England Biolabs, Beverly, MA, USA) and 2 U of *RsaI* enzyme (New England Biolabs, Beverly, MA, USA). Specifically, the digestion was conducted at 37°C for 2 h on a 30 µL volume composed as following: 20 µL of PCR products, 0.3 µL of BSA 100 µl/mL, 3 µL of 1× buffer (which was a solution composed of 10 mM of Tris-HCl, 50 nm of NaCl, 10 mM of MgCl2, 1 mM of dithiothreitol, pH 7.9, for *MnlI*, and 10 mM of Bis-Tris-propane-HCl, 10 mM of MgCl2, 1mM of dithiothreitol, pH 7.0, for *RsaI*). Subsequently, a deactivation process at 65°C for 20 min was carried out. The fragments thus obtained were separated by electrophoresis on 4% Agarose gel (GellyPhor, Euroclone, UK). A 50 bp DNA marker was also run with the digested fragments (Invitrogen, Carlsbad, CA, USA). Genotyping was conducted on all the samples.

Statistical analysis

Allele and genotype frequencies were determined through a direct count of the observed genotypes. The chi-squared test was used to determine Hardy-Weinberg equilibrium of the mutation (Genepop 4.2).

Results

In all breeds involved in the study, the amplification of genomic DNA by PCR and the use of primers designed by Messer et. al resulted in the production of 824 bp fragments (Messer et al., 1997).

Following digestion with the *MnlI* enzyme, the formation of 8 fragments with a length of 219, 36, 67, 236, 22, 28, 82, and 134 bp, respectively, was evidenced. None of the fragments were found to be polymorphic. The cleavage sites were located at 219, 255, 322, 558, 580, 608, and 690 positions, respectively. For this reason, all goats showed an M/M genotype (Table 2).

Table 2. Allelic and genotypic frequencies in the analyzed goat breeds.

Breed	M	m	M/M (%)	M/m (%)	m/m (%)
Frisa	1.00	0.00	100	0	0
Orobica	1.00	0.00	100	0	0
Lariana	1.00	0.00	100	0	0
Camosciata delle Alpi	1.00	0.00	100	0	0

Digestion of the 824 bp amplified fragment with the restriction enzyme *RsaI* resulted in the production of 5 fragments of 53, 267, 23, 411, and 70 bp length, respectively. Again, none of the fragments were polymorphic. The cleavage sites identified with the *RsaI* enzyme were 4, at position 53, 320, 344, and 755, respectively. For this reason, all goats showed an R/R genotype (Table 3).

Table 3. Allelic and genotypic frequencies in the analyzed goat breeds.

Breed	R	r	R/R (%)	R/r (%)	r/r (%)
Frisa Valtellinese	1.00	0.00	100	0	0
Orobica	1.00	0.00	100	0	0
Lariana	1.00	0.00	100	0	0
Camosciata delle Alpi	1.00	0.00	100	0	0

Discussion

The fragment of 824 bp found in all goat breeds following the amplification of exon 2’s genomic DNA is consistent with what has already been reported since the first investigation on the sheep genome (Messer et al., 1997) and followed by the few investigations on the goat species (Carcangiu, Vacca, et al., 2009; Chu et al., 2007; Korkmaz Agaoglu et al., 2015; Migaud et al., 2002), using the primers by Messer et al. (Messer et al., 1997).

The MTNR1A gene showed the same cleavage sites for *MnlI* and *RsaI* enzymes in Frisa, Orobica, Lariana, and Camosciata delle Alpi goat. Specifically, our results showed the existence of 7 cleavage sites for *MnlI* and 4 for *RsaI*. That is in agreement with what reported in previous studies both on sheep and goat (Carcangiu, Mura, et al., 2009; Carcangiu, Vacca, et al., 2009; Korkmaz Agaoglu et al., 2015; Migaud et al., 2002). That was only partially consistent with the study by Chu et al. which found just 6 cleavage sites for *MnlI* in 5 Chinese native breeds and a cosmopolitan goat breed (Chu et al., 2007). Regardless, in our study, none of the cleavage sites was polymorphic.

Regarding the fragments produced by *MnlI* digestion, our findings are consistent with what was reported by Migaud et al. (Migaud et al., 2002), which conducted the same experimental plan on Camosciata delle Alpi and Creole goat. Interestingly, also in that case, no polymorphic cleavage sites were reported in either breed, despite the former is a seasonal estrous breed, while the latter a year-round estrous breed, and differences among the two breeds were based on results in sheep species. The same unexpected result in goat was also obtained by Chu et al. and Carcangiu et al. (Carcangiu, Vacca, et al., 2009; Chu et al., 2007). In sheep species, a polymorphism at g.17355452 position was found following *MnlI* enzymatic digestion, which showed a strong link with reduced reproductive seasonality in different Mediterranean breeds. That was shown to be associated with a mutation at position g.17355358 which led to an amino acid substitution of Val to Ile, causing a modification in the melatonin signal transmission (Starič et al., 2020).

Concerning the polymorphism identified by the restriction enzyme *RsaI*, in our study, none of the 4 cleavage sites were polymorphic. Despite the sites identified are in concordance with what has been reported in the literature for the goat species (Carcangiu, Vacca, et al., 2009; Chu et al., 2007; Korkmaz Agaoglu et al., 2015), the absence of any polymorphism was an unexpected result. In fact, Chu et al. reported the presence of a polymorphic cleavage site following digestion with the above-mentioned enzyme (Chu et al., 2007). The allele associated with the presence of the cleavage site was termed R, while the absence of it was represented by the letter r. In the study by Chu et al., six goat breeds were considered, two of which were year-round estrous. The latter breeds showed an association with the RR genotype, while native Chinese breeds with marked reproductive seasonality were as-sociated with an Rr genotype. No goats showed rr genotype, from which a low frequency of the r allele in the goat species is assumed (Chu et al., 2007). The same results were also found in two different Turkish local goat breeds, but none of them showed a rr genotype (Korkmaz Agaoglu et al., 2015). Subsequently, an investigation by Carcangiu et al. confirmed the presence in the goat species of the polymorphic site identified at position 53 by the restriction enzyme *RsaI* (Carcangiu, Vacca, et al., 2009). In this study, four cosmopolitan goat breeds (i.e., Saanen, Camosciata delle Alpi, Maltese, and Nubiana), primarily reared for milk production, were examined in addition to the Sarda breed. The Sarda goat is a local Italian breed raised for the production of milk, which is then processed for traditional dairy production, although unlike cosmopolitan breeds, the Sardahas been less subject to selection by humans. The results showed the presence of the r allele exclusively in the Sarda breed, in association with a more marked reproductive seasonality (Carcangiu, Vacca, et al., 2009). For these reasons, considering the marked reproductive seasonality of the breeds analysed in our investigation, given also their origin in regions at higher latitudes than the Sarda breed, and the low artificial selection for reproductive activity (at least in the Italian local breeds enrolled), the absence of polymorphism with respect to digestion with the restriction enzyme *RsaI* represents a most unexpected result. Camosciata delle Alpi is an exception, being our results consistent with the study of Carcangiu et al. (Carcangiu, Vacca, et al., 2009). One hypothesis, mentioned by Carcangiu et al. [26], is that the absence of polymorphism may be linked to the macrogroup of goat breeds that evolved during human migrations. Specifically, breeds of the Alpine-European strain would appear to show no polymorphism, as confirmed by our study, whereas breeds belonging to the Mediterranean-African (Carcangiu, Vacca, et al., 2009) or Asian-Middle Eastern (Chu et al., 2007; Korkmaz Agaoglu et al., 2015) strain do.

Conclusions

In conclusion, our results showed the absence of polymorphism of the melatonin receptor gene MTNR1A identified by the restriction enzymes *MnlI* and *RsaI*, in the 3 Italian goat breeds (i.e., Frisa, Orobica, and Lariana) and in the transboundary goat breed Camosciata delle Alpi. This represents an unexpected result for the above-mentioned reasons. Therefore, to better investigate these aspects and to confirm our results, future studies are needed. In addition, future research studies should encompass a greater number of animals, despite the reduction in animals bred due to the progressive abandonment by farmers of the local breeds involved in our study. Moreover, further investigations on other autochthonous goat breeds of the peninsula, which are traditionally raised at different latitudes, could provide valuable insight into the role of the MTNR1A gene polymorphism in regulating the reproductive seasonality. Further analyses, including genetic investigations, could help broaden knowledge in this area. Finally, parallel analyses conducted on the ancestor of the domestic goat, i.e., *Capra aegagrus*, could be very interesting, following the example of what has already been done for the sheep species on *Ovis Gmelini Musimon*, a feral subspecies of the primitive domestic sheep (Carcangiu et al., 2010).

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3.3 COLOSTRUM QUALITY IN DIFFERENT GOAT BREEDS REARED IN NORTHERN ITALY

The data shown in this chapter refer to the already published paper: Agradi, S., González-Cabrera, M., Argüello, A., Hernández-Castellano, L. E., Castro, N., Menchetti, L., Brecchia, G., Vigo, D., Tuccia, E., & Curone, G. (2023). Colostrum Quality in Different Goat Breeds Reared in Northern Italy. Animals, 13(19), 3146. https://doi.org/10.3390/ani13193146

Introduction

Autochthonous livestock breeds are important as genetic resources of variability (Battaglini et al., 2014; B. Scherf & Pilling, 2015). Italy, due to its peculiar and diversified pedogeographical characteristics, has given rise over the centuries to the selection of numerous breeds (Bigi & Zanon, 2020; Curone et al., 2018, 2019): nearly 40 goat breeds have been recognized in the constitution of a Genealogical Book (ASSONAPA Associazione Nazionale Della Pastorizia, 2023). Despite their valuable multifunctional role, including the maintenance of biodiversity, most of the local caprine breeds are facing extinction. This is due to the progressive abandonment of these rustic but low-productive goats in favor of cosmopolitan breeds which are more productive and suitable for intensive farming (Agradi et al., 2022; FAO, 2023). For this reason, it is essential to study the physiology and ethology as well as the productive characteristics of the autochthonous breeds, to understand their adaptive strategies, and to encourage their use and conservation.

One of the most essential physiological mechanisms for the survival and development of goat kids is based on colostrum intake during the first hours of life. Colostrum is the first secretion of the mammary gland that the mother secretes after parturition. It provides vital nutrients to newborns which enhance their natural defenses and regulates immune response, gut microbiota, and growth and repair of tissues (Mann, Curone, Chandler, Moroni, et al., 2020; Mann, Curone, Chandler, Sipka, et al., 2020; Uruakpa et al., 2002). In fact, the molecules and immune cells contained in colostrum are very important in allowing the transfer of passive immunity and the creation of an initial protective barrier in the newborn's intestine and other mucus membranes (Uruakpa et al., 2002). These, however, are only some of the main functions performed by colostrum. In goats, colostrum composition has been investigated since 1840 in different breeds worldwide (Bergman & Turner, 1937). Studies have characterized the colostrum of German, Swiss (Kessler et al., 2019; Rudovsky et al., 2008), Spanish (Arguello et al., 2006; Moreno-Indias et al., 2012; Romero et al., 2013; Sánchez-Macías et al., 2014), and cosmopolitan breeds (Keskin et al., 2007; Levieux et al., 2002; M. Yang et al., 2015). On the other hand, Italian breeds have been neglected. Just a few studies have analyzed the colostrum of the Garganica goat, an indigenous breed of Southern Italy, and only as regards its oligosaccharides content (Claps et al., 2014, 2016). In general, goat colostrum is characterized by a dry matter above 20% with a high and variable percentage of fat (on average 8%) mainly constituted by short-chain fatty acids which are easily digested and absorbed by the newborn. In addition, it is also characterized by a high percentage of protein (usually above 10%) with a high biological titer and a relatively low percentage of lactose (around 2%). Not only does colostrum provide energy to the newborn mammal, but it also contains very important bioactive components that play a key role in passive immune transfer. The particular anatomical conformation of the goat placenta does not allow a sufficient transfer of immune components, mainly immunoglobulins, from the does to the fetus. Therefore, kids are born agammaglobulinemic and unable to cope with possible infections (Zhou et al., 2023). Thus, ruminants' colostrum is particularly rich in immunoglobulins (Ig), which make up one-third of the total colostrum proteins. In goat colostrum, IgG is the main immunoglobulin accounting for 90% of the total immunoglobulins, followed by IgM (6.0%) and finally IgA (3.7%) (Rudovsky et al., 2008). In addition to these protein molecules, other factors such as vitamins, hormones, growth factors, cytokines, enzymes and bioactive peptides, and immunocompetent cells act as adjuvants to the kid's immune system (Menchetti et al., 2018; Xu et al., 2021; Zhou et al., 2023). These include lactoferrin, an iron-binding glycoprotein, which makes metal ions unavailable to pathogens, inhibiting their growth. It is one of the most emphasized immune-stimulating factors of the bovine colostrum (Menchetti et al., 2016, 2020), but its content in the goat colostrum is still little known (Hiss et al., 2008; Rachman et al., 2015). The quality of colostrum may depend on various factors. Parity and litter size could influence it, but findings are still controversial (Arguello et al., 2006; Keskin et al., 2007; Kessler et al., 2019; Levieux et al., 2002; Moreno-Indias et al., 2012; Romero et al., 2013; Rudovsky et al., 2008; Sánchez-Macías et al., 2014; X. Y. Yang et al., 2009; Zhou et al., 2023), while regarding the farming system, studies have been carried out only in cattle (Puppel et al., 2019). Moreover, variations in the colostrum composition occur not only according to the species, but also to the breed, and, to our knowledge, there is no information concerning the local breeds of Northern Italy.

Specifically, within the zootechnical context of Northern Italy, the goats most commonly reared for milk and cheese production are two cosmopolitan breeds, the Camosciata Alpina or Alpine and the Saanen goats. These transboundary breeds are usually farmed with an intensive or semi-intensive system, which enables the genetic base artificially selected for better production performance to be fully exploited. In these contexts, the environment where the animals are bred, and the administered diet are controlled and standardized. The local breeds, although in the minority, are still widespread in the pre-Alpine and Alpine areas in small flocks (Sandrucci et al., 2019). Frisa Valtellinese, Orobica, and Lariana are among the most important autochthonous breeds in Northern Italy, where they are used for the production of typical products, especially fresh or seasoned cheeses, and kid meat. These breeds are farmed according to the traditional system, based on a close environment-(domestic) animal-human link. In fact, during the winter months, the animals are housed indoors and fed with hay, while usually in spring and autumn, intermittent grazing is carried out in the areas surrounding the farms. Finally, in the summer months, the traditional vertical transhumance is performed, whereby the flocks are moved to high altitudes to graze on alpine pastures (Nicoloso et al., 2015). Few studies have involved the caprine heritage of these areas, especially as regards local breeds. Defining the quality of colostrum could be significant for understanding the nutritive concentration capacities and immunological status of the females, and, as a consequence, the future health status and growth of the newborns (M. Yang et al., 2015). Moreover, colostrum can be considered a nutraceutical that can potentially provide beneficial effects on human (Florén et al., 2006; Otto et al., 2011; Sponseller et al., 2015) and animal health (Agradi et al., 2023; Balan et al., 2019; Serra et al., 2023). Thus, studies on the composition of the goat colostrum could be also useful to produce supplements that could be used in human and animal medicine.

We hypothesize that the composition of colostrum is influenced by breed and that local breeds may have similar characteristics, due to the similar farming system and evolutionary processes, that distinguish them from the cosmopolitan one. The composition of colostrum could be thus a trait related to the goat's adaptability and contribute to the greater rusticity of the autochthonous breeds. This study aimed to characterize the colostrum quality in three different local goat breeds of Northern Italy (i.e., Frisa Valtellinese, Orobica, and Lariana), and a cosmopolitan one (i.e., Camosciata delle Alpi or Alpine), reared under traditional semi-extensive and intensive systems, respectively, both from a basic composition (fat, protein, lactose, and total solids percentages) and immunological (IgG, IgM, and lactoferrin concentrations) point of view.

Materials and Methods

Animals Enrolled

In this investigation, 120 adult female goats were enrolled (i.e., Frisa, *n* = 30; Orobica, *n* = 30; Lariana, *n* = 30; and Camosciata delle Alpi, *n* = 30).

The Frisa or Frontalasca is a dual-purpose goat (Figure 1a) native to the Rezzalo valley, in North Lombardy. It is mainly reared in Valtellina, Malenco Valley, Masino Valley, Valchiavenna, Valcamonica, Bergamo Valleys, and in the Lario area (ARAL (Associazione Regionale Allevatori

Lombardia), n.d.). Its most relevant morphologic characteristics are its black coat (short or medium length hair) with white stripes on both sides of the head up until the ears; its white hair on the ventral part of the abdomen, on the limb distal extremities, and under the tail; and its ibex type horns (even if polled subjects may be seen) (Crepaldi et al., 2001). It is a large-sized goat, well-proportioned, and with a strong constitution that also allows it to exploit the most difficult pastures (ARAL (Associazione Regionale Allevatori Lombardia), n.d.). The Anagraphic Registry was instituted in 1997. In 2016, the registered population was 785 heads in 51 farms (*ASSONAPA Associazione Nazionale Della Pastorizia*, 2023). This goat breed is mainly reared to produce typical cheeses and meat, especially the traditional Valchiavenna goat Violino, which is made by seasoning the legs and shoulders of adult goats.

(a) (b)

(c) (d)

Figure 1. Frisa Valtellinese (**a**), Orobica (**b**), Lariana (**c**), and Camosciata delle Alpi goats (**d**) in their respective breeding environment in different times of the year.

The Orobica or Valgerola goat (Figure 1b) is an autochthonous breed reared in the Lombardy region in Northern Italy. Its origin is unknown, even if some hypotheses about a Southern Italian provenance (also supported by the oral tradition of old local breeders) have been formulated (Ajmone-Marsan et al., 2001; Crepaldi et al., 2001). Nowadays, this breed can be found in the Orobic Prealps in small farms mainly in the Sondrio, Como, Lecco, and Bergamo provinces and, in particular, in Val Gerola, Valsassina, Upper East Lario, and Val Brembana (ARAL (Associazione Regionale Allevatori Lombardia), n.d.). Morphologically, it is a medium size goat, well-proportioned and adapted to the mountain pasture (Chiatti et al., 2007). The animal is characterized by the presence in both sexes of long horns with a slight helical twist and by four long hair coats which differ in color and pigment distribution (Farinel, Marin, Nigru and Camosch) (Chiatti et al., 2007), characteristics that allow their differentiation from other alpine goat breeds and that testify to the probable different origin of Orobica (Crepaldi et al., 2001). The Genealogical Book was activated in 1992, and at the end of 2016, the registered population was 1294 heads in 93 farms (ASSONAPA Associazione Nazionale Della Pastorizia, 2023). It is a dual-purpose breed, with particularly important cheese production. Indeed, the milk is mainly utilized to produce Valtellina's Bitto cheese, Storico Ribelle cheese (both with at most 10% goat milk), and Orobica goat cheeses (100% Orobica goat milk) (Associazione Formaggi Principi delle Orobie, 2015).

The Lariana or di Livo goat (Figure 1c) is another Lombard autochthonous breed mainly reared in the Livo Valley and Western Lario whose origin is unknown (ARAL (Associazione Regionale Allevatori Lombardia), n.d.). It is classified as a traditional or primary population because of its highly phenotypic polymorphism (Crepaldi et al., 1999). It is a medium–large size goat with a strong constitution (ARAL (Associazione Regionale Allevatori Lombardia), n.d.). Being that this breed has not undergone a selection for the phenotypic characteristics, morphologically it shows a wide variety of coat colors, hair lengths, and horn types (Crepaldi et al., 1999). The Anagraphic Registry was activated in 2001, and at the end of 2016, the registered population was 615 heads in 50 farms (ASSONAPA Associazione Nazionale Della Pastorizia, 2023). It is mainly bred to produce cheeses and meat.

The farming system is the traditional one for all three of these local breeds (Nicoloso et al., 2015). They are very rustic animals, adapted to the Alpine environment where they have been selected for centuries.

The Camosciata delle Alpi or Alpine goat (Figure 1d) is a transboundary dairy breed native to Switzerland, from the cantons of Bern, Freiburg, Glarus, and Graubünden in a mountainous region. It is bred in different countries of the world, mainly in Europe but also in other continents such as North America. This breed is a medium–large size goat, with a typical short hair fawn coat, black dorsal stripe and extremities of limbs, and a characteristic facial mask. They have ibex-type horns both in males and females. In Italy, the Genealogical Book was activated in 1973. To date, the breed is subjected to accurate genetic selection to improve quantitative and qualitative milk and cheese production (ASSONAPA Associazione Nazionale Della Pastorizia, 2023). Artificial selection in the past decades has led, as in the bovine sector, to the production of less rustic animals, which are less suitable for the semi-extensive farming system and to the exploitation of extreme environments such as the alpine one.

The goats enrolled in the study belonged to 4 different farms according to the breed. The Frisa farm was in Val Bregaglia in the Sondrio province, the Orobica farm was at the beginning of Valtellina in the Sondrio province, and the Lariana farm was in Valle Albano in the Como province. The traditional farming system implied indoor housing from mid-November till mid-May with administration of polyphyte hay *ad libitum*. During the spring and autumn months, at the beginning and at the end of the indoor housing period, the goats were left free to graze in the vicinity of their respective farms for a limited number of hours/day in order to get used to the fresh forage before vertical transhumance in spring and to get used to a hay-based diet in winter. Vertical transhumance was performed in mid-May to the respective alpine pasture for all farms. The respective alpine pasture areas were located in Val Bregaglia at around 1400–2000 m.s.l., in Val Masino at around 1600–2700 m.s.l., and in Valle Albano at around 1100–1600 m.s.l. All 3 farms kept between 50 and 100 lactating goats only for the production of cheese under a semi-extensive system, following the alpine traditional farming system mentioned above. The Camosciata delle Alpi farm was in the Lodi province in South Lombardy and bred around 100 lactating goats under an intensive system for both the production of milk and cheese. They received a unified diet during the dry period (feed consisted of polyphyte hay, maize, soya, hulled sunflower seed meal, extruded linseed, cane molasses, carob, mineral vitamin corrector), specially designed to reach the energetic requirements according to the physiological stage of the animal. For all breeds, reproduction occurred with natural mating, and the birth season took place from the last week of December till the last week of February 2021. All the goats were milked twice a day after birth, with a milking machine for Camosciata delle Alpi and Frisa during the indoor housing period, and by hand for Frisa during the alpine pasture period and for Orobica and Lariana for the whole lactation. Lactation lasted around 8 and 7 months for Camosciata delle Alpi and Frisa, respectively, while it lasted just 6 months for Orobica and Lariana.

All the goats enrolled in the study were submitted to a clinical evaluation before starting the investigation, and only healthy animals were selected. Since major differences related to the parity order may arise among primiparous and multiparous (Higaki et al., 2013; Romero et al., 2013), nulliparous goats were not included in this study to minimize the possible bias. The parity order ranged from 1st to 10th (not considering the birth of the kidding season we investigated), while the mean (and standard deviation) age of enrolled goats was 5.6 ± 2.7, 4.9 ± 2.1, 5.4 ± 3.1, and 3.0 ± 1.1 years for Frisa, Orobica, Lariana, and Camosciata delle Alpi goats, respectively. Litter size ranged from 1 to 3 kids (Table S1).

Colostrum Collection

Within 6 h of parturition, before kids began suckling, 50 mL of colostrum were collected by hand milking from both mammary glands in 50 mL Falcon® tubes (Corning, Corning, NY, USA) from 30 adult goats per breed. The samples were immediately frozen at −20 °C and stored until analysis. The collection of colostrum samples from live animals was performed in respect of animal welfare according to current legislation. The study was conducted with the approval of the Institutional Animal Care and Use Committee of Università degli Studi di Milano (Permission OPBA_04_2021). Samples that were insufficient in quantity for analysis, and samples derived from goats that developed any pathology during the peri-partum period, were discarded. For these reasons, a total of 102 samples were analyzed (i.e., 29, 18, 27, and 28 samples for Frisa, Orobica, Lariana, and Camosciata delle Alpi, respectively).

Colostrum Analysis

The basic composition (i.e., fat, protein, lactose, and total solids percentages) was determined by MilkoScan Mars™ (FOSS Analytical A/S, Hillerød, Denmark). For this analysis, colostrum samples were diluted 1:1 (*v/v*) using saline solution. Immunoglobulins G, IgM, and lactoferrin concentrations were measured using ELISA commercial kits (Bethyl Laboratories, Montgomery, TX, USA and MyBioSource Inc., San Diego, CA, USA, respectively). All determinations were carried out in duplicate. The intra- and inter-assay coefficients of variation (CV) were <6% and <5% for IgG, <3% and <5% for IgM, and <10% and <10% for the lactoferrin kit.

Statistical Analysis

Diagnostic graphs, Kolmogorov-Smirnov, and Levene's tests were used to verify assumptions. Two outliers were eliminated (i.e., 1 for IgG and 1 for fat), while lactoferrin was ln-transformed to improve data distribution. Descriptive statistics were used to present variables as means and standard error of the mean (Agradi et al., 2022). Then, the effect of breed on colostrum composition was first investigated using ANCOVA via Generalized Linear Models (GLMs) Univariate procedures including the parity order and litter size as covariates. The assumption of independence of the covariate and breed effect was verified by checking whether breeds differ in parity order and litter size (Field, 2013) using, respectively, Kruskal-Wallis and Fisher's exact tests. These tests showed the lack of independence between breed and litter size since the Camosciata had a greater proportion of twins than local breeds ($p < 0.001$; Table S1). Therefore, the litter size could not be included as a covariate in the ANCOVAs. On the other hand, GLMs including parity order as covariate, breed as fixed factor, and colostrum components as a dependent variable showed that parity order was never significant ($p > 0.1$ for all the variables). Finally, we decided to exclude them and use the classic procedures of the 1-way ANOVA where only the breed effect was evaluated. Welch's F was used for IgG, IgM, ln-lactoferrin, protein, and total solids as the homogeneity of variance assumption was not met. To highlight the differences between the cosmopolitan and local breeds and farming systems, the contrast "Camosciata vs. [(Frisa + Orobica + Lariana)/3]" was planned. Moreover, pairwise comparisons were performed using Sidak correction.

Subsequently, breeds were categorized into a dichotomous variable according to the breed type (local and cosmopolitan) and farming system (traditional semi-extensive and intensive systems). The two groups included Camosciata (ID = 1) and Frisa, Orobica, and Lariana (ID = 2). Then, all colostrum components were included in a discriminant analysis (DA). DA is a multivariate technique intended to identify the colostrum components that distinguish the two groups (i.e., local breeds from the cosmopolitan one) and quantify their relative importance (Agradi et al., 2020; Garson & David, 2012). The coefficients of the discriminant function (Df) were adjusted for group size. The relative importance of each component in classifying the goat's group (i.e., cosmopolitan vs. local breeds) was evaluated by using Wilks' lambda (λ, the smaller the Wilks' λ score, the more important the variable to the Df) and by the structure coefficient (which assesses the importance of each independent variable's unique contribution to the discriminant function) (Garson & David, 2012).

Statistical analyses were performed with SPSS 25.0 (SPSS Inc., Chicago, IL, USA) and statistical significance occurred when $p < 0.05$.

Results

The breed effect on colostrum composition was significant for all components analyzed except IgM ($p \leq 0.001$; Table 1). Results of pairwise comparisons showed that Orobica had the lowest values of protein and total solids along with the highest values of lactose ($p < 0.05$); conversely, Lariana had the lowest content of lactose and the highest content of total solids ($p < 0.05$). Frisa had the highest content of both IgG and lactoferrin; Camosciata, on the contrary, showed the lowest values for these components ($p < 0.05$). As regards the fat percentage, Camosciata and Orobica had the lowest values while Lariana had the highest ($p < 0.05$).

Table 1. Colostrum components in Camosciata and local breeds. Data are means, standard errors of the mean (SEM), and range (minimum and maximum). Lactoferrin was analyzed after logarithmic transformation, but raw data are shown. Last column reports the *p*-value for the planned contrast Camosciata vs. local breeds.

Parameter	Breed								p Value of ANOVA	p Value of the Contrast
	Camosciata		Frisa		Lariana		Orobica			
	Mean ± SEM	Range (Min–Max)	Mean ± SEM	Range (Min–Max)	Mean ± SEM	Range (Min–Max)	Mean ± SEM	Range (Min–Max)		
Fat (%)	6.92a ± 0.53	2.40–12.62	8.33ab ± 0.52	4.66–13.06	10.18b ± 0.66	3.80–16.06	7.13a ± 0.59	3.46–10.94	0.001	0.018
Protein (%)	14.16b ± 0.59	7.10–20.44	15.43b ± 0.81	8.14–23.34	16.20b ± 0.72	7.54–23.86	10.77a ± 1.07	3.52–19.78	0.001 *	0.969
Lactose (%)	2.50b ± 0.12	1.12–3.50	2.42ab ± 0.16	0.22–4.08	1.87a ± 0.17	0.14–3.18	3.16c ± 0.17	1.34–4.30	<0.001	0.959
Total solid (%)	26.37ab ± 0.62	20.90–32.84	28.76bc ± 0.99	20.10–38.36	30.73c ± 1.02	16.68–39.36	24.11a ± 1.29	15.66–35.44	0.001 *	0.100
IgG (mg/mL)	74.75a ± 4.12	34.21–106.38	100.90c ± 1.56	75.70–113.30	93.07bc ± 2.71	64.29–108.00	80.27ab ± 5.57	37.38–105.58	<0.001 *	0.001
IgM (mg/mL)	1.40 ± 0.16	0.22–2.87	1.85 ± 0.12	0.29–3.30	1.57 ± 0.13	0.41–2.75	1.44 ± 0.24	0.05–3.19	0.122 *	0.243
Lactoferrin (µg/mL)	763.10a ± 76.31	435.68–1920.03	1781.31b ± 168.69	345.35–3232.93	1148.00a ± 179.03	327.94–3321.82	1132.41ab ± 153.08	425.56–2759.11	<0.001 *	0.001

* Welch statistic. Values followed by the same letter in each row do not differ significantly ($p < 0.05$); multiple comparisons with Sidak correction.

Planned contrast “Camosciata vs. local breed” indicated that the IgG ($p = 0.001$), lactoferrin ($p = 0.001$), and fat ($p = 0.018$) concentrations of the Camosciata were lower than the average of the local breeds (Table 1).

Differences between cosmopolitan and local breeds, and consequently between different farming systems, were further investigated through discriminant analysis (DA). The DA produced a model capable of discriminating Camosciata from local breeds through the characteristics of their colostrum (model Wilks’ $\lambda = 0.559$, $p < 0.001$). Figure 2 shows the variables included in the DA in order of importance according to their structure coefficients and Wilks λ. The component that contributed the most to this discrimination was IgG (p for $\lambda < 0.001$), followed by lactoferrin concentration (p for $\lambda = 0.002$) and fat percentage (p for $\lambda = 0.009$). The figure also shows the coefficients, which are all positive except for the lactose content. The group centroids (−1.532 for Camosciata and 0.503 for local breeds) indicated that local breeds were distinguished by a greater content of IgG, lactoferrin, fat, IgM, and total solids than Camosciata (lactose and protein had $\lambda > 0.99$ and, thus, contributed little to the discrimination).

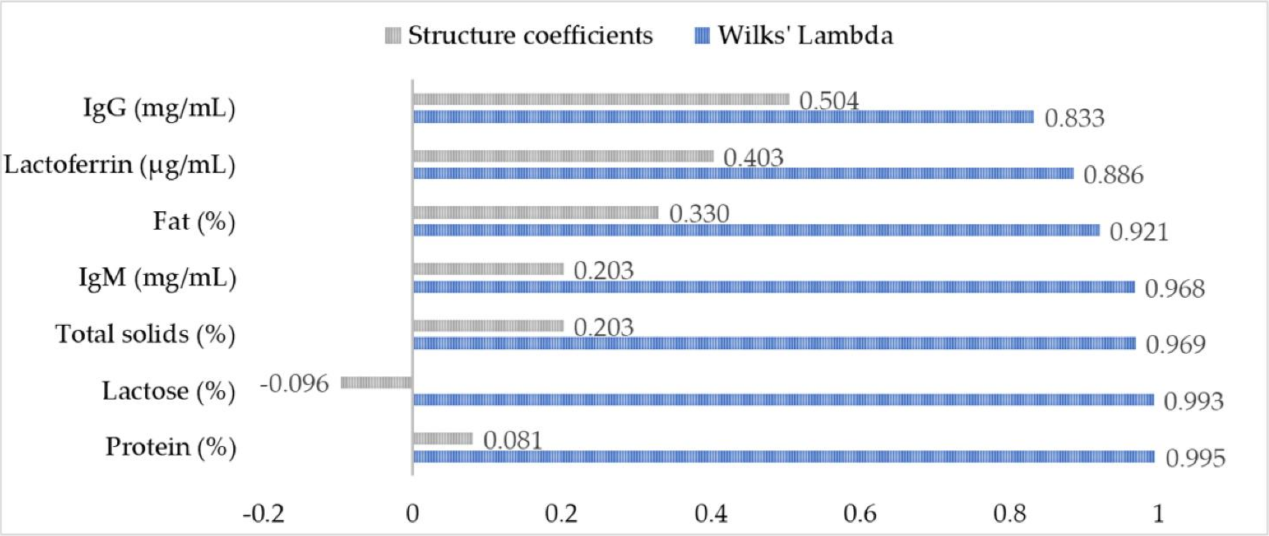

Figure 2. Discriminant analysis. Structure coefficients and Wilks' lambda of colostrum component in the discriminant analysis to discriminate Camosciata from local breeds. The variables are shown in order of importance in the discrimination.

Discussion

This study describes for the first time the colostrum composition of goat breeds reared in Northern Italy. The values we found for fat, protein, lactose, and total solids are in agreement with the literature (Arguello et al., 2006; Keskin et al., 2007; Kessler et al., 2019; Moreno-Indias et al., 2012; Romero et al., 2013; Rudovsky et al., 2008; Sánchez-Macías et al., 2014; X. Y. Yang et al., 2009). However, this study also shows that significant differences in the composition of the colostrum among goat breeds exist. Specifically, regarding the chemical composition of the colostrum, Lariana showed the highest percentage of fat and total solids, while it showed the lowest percentage of lactose. On the other hand, Orobica had the lowest percentage of fat, total solids, and protein but the highest percentage of lactose. The pattern of those results was expected, considering that lactose is an osmotic active molecule. Thus, a higher percentage of it corresponds to a higher water recall and, as a consequence, a higher dilution of the other basic components of colostrum, and vice versa (Fox et al., 2015). Our results also suggest that goat breeds which have a more pronounced meat aptitude (i.e., Frisa and Lariana) present a higher concentration of colostrum components (i.e., fat, protein, and total solids) than those with a more pronounced dairy aptitude (i.e., Orobica and Camosciata). Previous studies showed conflicting results. While Kessler et al. found few differences in colostrum composition related to the purpose for the goats, they also reported similar results to the present study for sheep (Kessler et al., 2019). In particular, these authors found that milk-type sheep breeds had lower fat and protein with concomitantly higher lactose percentages than the meat-type breeds. In goats, they only highlighted a higher IgG concentration in animals kept for meat production than in dairy breeds (Kessler et al., 2019).

Similarly, in the present study, a significant difference was found in immunological parameters of the more rustic, autochthonous breeds reared with traditional systems and those of the cosmopolitan Camosciata delle Alpi. In particular, IgG is the parameter that best enables the differentiation between local and cosmopolitan breeds and thus between farming systems. The highest concentration was found in the Frisa goat; the lowest in the Camosciata. It should also be specified that in our study, total immunoglobulin G content was determined, as it was not possible to quantify the IgG subclasses (due to the lack of specific kits). The values obtained are of the same order of magnitude as those reported in the scientific literature. However, we can notice that all four breeds were in the upper limit of the range of IgG previously observed in goat colostrum (Arguello et al., 2006; Kessler et al., 2019; Levieux et al., 2002; Moreno-Indias et al., 2012; Romero et al., 2013; Sánchez-Macías et al., 2014; X. Y. Yang et al., 2009). In fact, Kessler et al. (Kessler et al., 2019), who analyzed the colostrum of several Swiss and German goat breeds, observed an average IgG value between 4.8 and 75.0 mg/mL, with the Boer (i.e., a goat reared for meat production) showing the highest concentration. Interestingly, Levieux et al. (Levieux et al., 2002) reported a highly variable range for the Camosciata (from 19.9 to 94.5 mg/mL) but this range includes the values we found for that breed.

In the present study, the IgM concentrations were also determined. They have antimicrobial properties (e.g., neutralization of viruses and agglutination of microbes) and anti-inflammatory extracellular and intracellular immune exclusion, inhibiting adherence and invasion of mucosal epithelia. Thus, IgMs can exert the first defense action in the intestinal epithelium of the newborn (Hurley & Theil, 2011). The scarcely available scientific literature on this molecule reports higher mean values than those found in this investigation (Moreno-Indias et al., 2012; Rudovsky et al., 2008; Sánchez-Macías et al., 2014). Since our determination techniques do not differ from previous studies, the disagreement may be due to differences in breeds (i.e., Majorera and Weiße Deutsche Edelziege goats) and farming systems. However, these immunoglobulins represent a minor proportion of the total immunoglobulins in goat colostrum in relation to IgG (Rudovsky et al., 2008). Moreover, we did not find any differences among the evaluated breeds.

Overall, compared to previous studies, we found higher IgG and lower IgM values. However, these findings do not appear to be associated. The mechanisms exploited by these two immunological molecules are different (Zhou et al., 2023), and there is no competition at the level of basal-apical transport in the alveolar cells of the mammary gland. Differences between breeds were instead found for lactoferrin concentrations. They showed the highest concentration in the Frisa goats and the lowest in Lariana and Camosciata delle Alpi. When compared to previous studies, our results exhibited much higher values, especially for goats with a more pronounced meat aptitude. Hiss et al. (Hiss et al., 2008) reported a lactoferrin concentration of 387 ± 69 µg/mL in the colostrum of German Improved Fawn and White goats, while Rachman et al. (Rachman et al., 2015) found a range of 156.4 to 207.4 µg/mL in Peranakan Etawah, Jawarandu, and Saanen goat crosses with Peranakan Etawah goats. In addition to breed and management differences, the varying analytical techniques used in these studies could also explain the differences in values. Regardless, in the present study, the more meat-aptitude goats also showed better results than the dairy ones in the case of the immunological quality of the colostrum. That is consistent with what was found by Kessler et al. (Kessler et al., 2019) regarding several Swiss and German goat breeds, and by Altvater-Hughes et al. focusing on cattle (Altvater-Hughes et al., 2022).

The differences between the Camosciata delle Alpi and local breeds were pointed out by planned contrasts and discriminant analysis (DA). In particular, the DA showed that an accurate statistical model can be built for their discrimination and that IgG was the component that mostly contributed to distinguish them, followed by lactoferrin concentration and fat percentage. This discrimination may also be related to the different nutritional management of the animals enrolled in the present study. Moreover, it could be explained by two different factors linked to the evolutionary processes of the breeds. First, Camosciata has a higher milk aptitude due to the artificial selection implemented in recent decades. This high level of milk production could have led to an increase in the lactose percentage and, consequently, to a decrease in the other colostrum components. Secondly, the local breeds have been more exposed to pathogenic noxious substances than intensively reared animals during their evolutionary history. They also have a lower milk production. These factors could enhance immune components and fat concentrations in their colostrum to ensure a higher probability of survival of their offspring (M. Yang et al., 2015).

Some limitations should be pointed out in the current study especially related to the many factors that could influence the composition of colostrum and which have not yet been well defined. For example, the Camosciata and the local breeds differed in their rearing and nutritional systems, and it is not possible to separate the genetic aspect from the management one. Future research could develop protocols in experimental stables where farming systems and diets can be standardized and identical for all breeds. On the other hand, our study provides information on the colostrum quality which truly characterizes the goats of the Italian livestock heritage as no modifications were introduced to the typical, currently applied farming systems. Instead, we tried to limit the bias due to the parity order by enrolling only multiparous goats. Among them, the preliminary analyses indicated no difference due to their parity, but further investigations should also include primiparous goats to better understand the role of this factor in influencing colostrum quality. Regarding the possible effect of the litter size, we have found that it is associated with the breed, and thus it was not possible to include it as a covariate in the statistical models (because it would have violated an assumption of analysis). On the other hand, the number of animals did not allow an analysis within each breed. This could represent a bias, and future studies will be necessary with a larger sample size. Finally, in further research, kids could be monitored to assess the association between colostrum quality and their growth performance.

Conclusions

In conclusion, this study has shown the existence of variability in colostrum quality among local and cosmopolitan breeds of goat which could probably be linked to the different farming systems and may be explained by their human-mediated selection and natural adaptation to the environment. However, even among the autochthonous breeds we found some significant differences, which might be ascribed to different aptitudes (meat or dairy). In general, the higher quality of colostrum produced by some local goats could be associated with hardiness and rusticity as it helps the growth and survival of the kids.

Supplementary Materials

Table S1. Litter size per breed.

Number of kids	**Breed**				**Total**
	Camosciata	**Frisa**	**Lariana**	**Orobica**	
1	5_a (17.9%)	18_b (62.1%)	22_b (81.5%)	15_b (83.3%)	60 (58.8%)
2	21_a (75.0%)	11_b (37.9%)	5_b (18.5%)	3_b (16.7%)	40 (39.2%)
3	2_a (7.1%)	0_a (0.0%)	0_a (0.0%)	0_a (0.0%)	2 (2.0%)
Total	28 (100.0%)	29 (100.0%)	27 (100.0%)	18 (100.0%)	102 (100.0%)

Each subscript letter denotes a subset of Breed categories whose column proportions do not differ significantly from each other at the 0.05 level.

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3.4 COMPARISON OF FEMALE VERZASCHESE AND CAMOSCIATA DELLE ALPI GOATS' HEMATOLOGICAL PARAMETERS IN THE CONTEXT OF ADAPTATION TO LOCAL ENVIRONMENTAL CONDITIONS IN SEMI-EXTENSIVE SYSTEMS IN ITALY

The data shown in this chapter refer to the already published paper: Agradi, S., Menchetti, L., Curone, G., Faustini, M., Vigo, D., Villa, L., Zanzani, S. A., Postoli, R., Kika, T. S., Riva, F., Draghi, S., Luridiana, S., Archetti, I., Brecchia, G., Manfredi, M. T., & Gazzonis, A. L. (2022). Comparison of Female Verzaschese and Camosciata delle Alpi Goats' Hematological Parameters in The Context of Adaptation to Local Environmental Conditions in Semi-Extensive Systems in Italy. Animals, 12(13), 1703. https://doi.org/10.3390/ani12131703

Introduction

The theme of livestock biodiversity in recent years is becoming increasingly important in the context of agriculture, rural development, and food and nutrition security (Scherf and Pilling 2015). According to FAO's "The State of the World's Biodiversity for Food and Agriculture" report, biodiversity is the variety of life at genetic, species, and ecosystem levels (FAO 2015). To date, livestock biodiversity is being lost at an alarming rate (Curone et al. 2019). For instance, according to this report, out of a total of 618 different goat breeds, just 306 were classified as not at risk, while the others were considered as at risk, with unknown risk level or extinct (FAO 2007). The high-yielding breeds are the most farmed at the world level and genetic selection has favored some desirable traits and the standardization of productions. However, this has caused a loss of intra- and inter-breed genetic variability (Biscarini et al. 2015) as well as a dramatic reduction in the number and consistency of local breeds. Local or autochthonous breeds are characterized by important features resulting from the close interaction of their genetic background and the environmental conditions where they have been living for centuries (Scherf and Pilling 2015). For example, in the DAD-IS (Domestic Animal Diversity Information System) 62 goat breeds adapted to mountainous terrain are recorded, 30 that are heat-tolerant, 7 humidity-tolerant, 14 cold-tolerant, and 20 adapted to water scarcity (Scherf and Pilling 2015). Moreover, the preservation and rational use of local breeds play a crucial role in maintaining biodiversity and valorization of local products (Agradi et al. 2020; Curone et al. 2018; Matassino n.d.). In Italy, the goat sector has a high level of biodiversity, with nearly 50 different local breeds (FAO 2022); the autochthonous breeds are farmed for their milk (Currò et al. 2019; Vacca, Stocco, Dettori, Pira, et al. 2018; Vacca, Stocco, Dettori, Summer, et al. 2018), meat quality and adaptive features, especially for the rusticity, frugality, fertility, and longevity (Currò et al. 2020; Di Trana et al. 2015). These features make local goat breeds the first choice in marginal areas where other domesticated species struggle to graze (such as mountains, wood, and foothill grazing), helping the preservation of the habitat and landscape integrity, reducing fire and flood risks, and promoting seedling establishment by reducing biomass accumulation (Ajmone-Marsan et al. 2001; Battaglini et al. 2014; De Haan, Steinfeld, and Blackburn n.d.).

For these reasons, it is of pivotal importance to take care of these animals' health in the optic of conservation strategies. Previous studies highlighted differences in the resistance/resilience against diseases and gastrointestinal parasites between cosmopolitan (e.g., Saanen and Camosciata delle Alpi) and local goat breeds (Alberti et al. 2014; Saddiqi and Jabbar 2011; Zanzani et al. 2019). Knowledge of hematological reference values of several goat breeds is still missing at a worldwide level. Different physiological adaptations of local breeds to specific environments can also be seen in the hematological parameters varying between one breed to another, as already described in many African (Njidda 2013; Tibbo et al. 2004) and Asian (Al-Bulushi, Shawaf, and Al-Hasani 2017; Mohammed Muayad et al. 2018; Osman, Al-Busaidi, and Johnson 2016; Shaikat et al. 2013) breeds. In Italy, the hematological profile has been investigated without specifying any reference intervals in Nicastrese (Castagna et al. 2017), Messinese (Zumbo et al. 2011), Girgentana, and Aspromontana (Piccione et al. 2014) breeds. Hematological reference values have been established only for a few Mediterranean goat breeds in one study (Aspromontana, Girgentana, Messinese, Maltese, and Argentata dell'Etna goats (Arfuso et al. 2016)). Moreover, it is fundamental to take into consideration the factors which could influence the hematological profile of a patient. In particular, it is now well known that the hematological parameters in goats undergo changes concerning many factors such as breed (Arfuso et al. 2016; Mohammed Muayad et al. 2018; Piccione et al. 2014), age (Antunović et al. 2020; Tibbo et al. 2004), sex (Mohammed Muayad et al. 2018; Ramprabhu et al. 2010), physiological/reproductive status (Azab and Abdel-Maksoud 1999; Mbassa and Poulsen

1991; Waziri, Ribad, and Sivachelvan 2010), season (Abdalla, Ibrahim, and Hassan 2009; Ghosh, Amaresh K Singh, and Haldar 2014; Habibu et al. 2017), environmental and management factors (Olayemi et al. 2009; Perumal et al. 2019; Ribeiro et al. 2018), nutrition (Solaiman et al. 2009), and diseases (Akinrinmade and Akinrinde 2012; Hristov et al. 2018; Sulaiman et al. 2010; Zaki et al. 2010). Based on these differences, it is necessary to establish for every breed appropriate physiological reference intervals that could be used to evaluate the physiological or pathological status of the animal.

Camosciata delle Alpi or Alpine goat (Figure 1a) is a dairy breed native to the mountainous cantons of Bern, Freiburg, Glarus, and Graubünden in Switzerland. However, it is farmed in many European (especially in France, Italy, and Germany) and non-European countries. In Italy, it is mainly reared in the Alpine regions, especially in Piedmont and Lombardy. The Alpine goat is medium-large size, originally well adapted to the climate and mountainous geographical conditions. It has undergone a strong selection for quantitative milk production, neglecting other characteristics such as rusticity and resistance/resilience to diseases. The Verzaschese, also known as the Nera di Verzasca or Verzasca goat (Figure 1b), is a double aptitude native breed of the Verzasca Valley in the canton of Ticino in southern Switzerland. It is raised in that area and in some provinces of Lombardy and Piedmont, in the North of Italy. It is a medium-large size goat and is characterized by high rusticity and resistance both to high and low temperatures. In Italy, the Verzaschese is one of the 46 autochthonous goat breeds of limited distribution for which the national association of pastoralism (AssoNaPa) keeps a herd book.

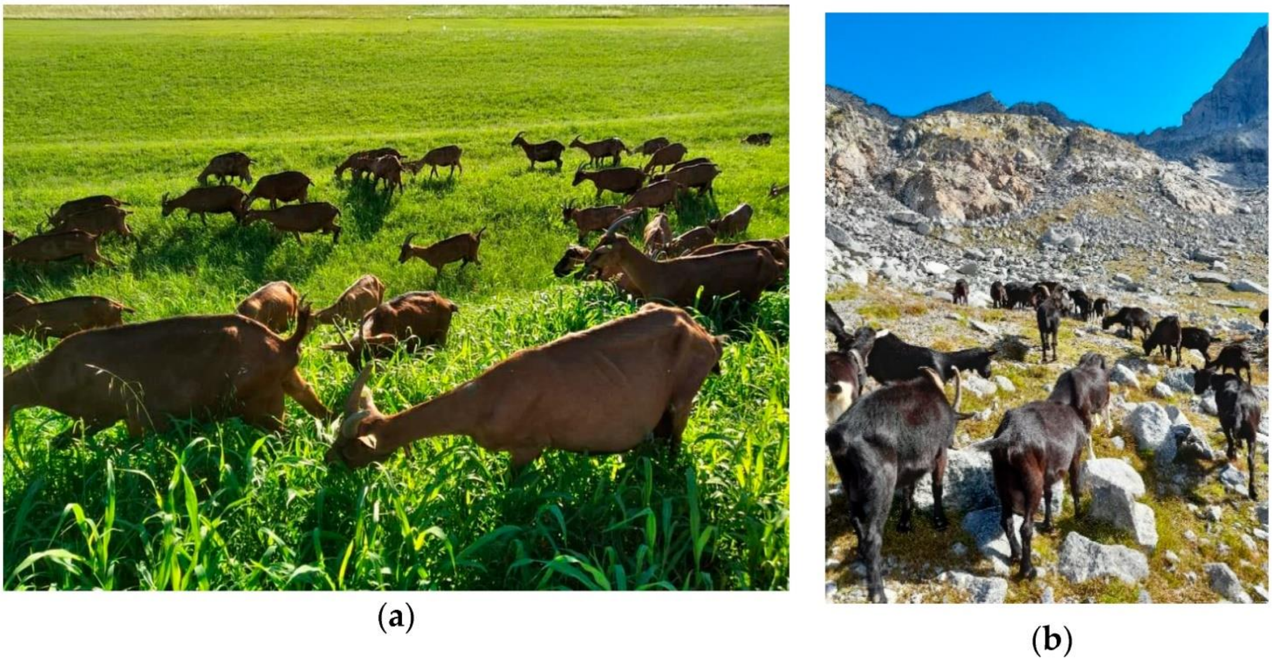
(a) (b)

Figure 1. Camosciata delle Alpi (**a**) and Verzaschese (**b**) goats during free grazing (by courtesy of Mrs. Paola Rossi and Mr. Franco Del Bondio, respectively).

To date, for both of these breeds, there is a lack of specific hematological reference values, and also the investigation of their complete blood cell count (CBC) in relation to age and season has never been reported in scientific literature. Just one study examined the variation in the hematological parameters through lactation in Camosciata delle Alpi goats (Antunović et al. 2013), but reference intervals were not established and the sample size, as well as observations, were limited. We hypothesized that the baseline hematological parameters of the cosmopolitan (i.e., Camosciata delle Alpi) and local breed (i.e., Verzaschese) goats could differ, probably reflecting their different adaptative capacities to the specific Western alpine mountainous environment and the farming system. We also hypothesized that these parameters could be influenced by age and season.

Thus, this study aimed at establishing hematological reference values in female subjects of two breeds (one cosmopolitan and the other local): the Camosciata delle Alpi and the Verzaschese goat, reared in the same conditions. A second part of the study investigated the influence of breed, age, and season on the hematological parameters.

Materials and Methods

Farm Conditions and Animal Selection

A total of 71 female goats of Camosciata delle Alpi (*n* = 37) and Verzaschese (*n* = 34) breeds were enrolled for this study (Figure 1).

All the animals were part of a flock reared in a unique structure under a semi-extensive farming system. Thus, the management conditions were the same for the two breeds. The farm was located at 980 m.s.l. (meters above mean sea level) in the mountains near Verbano Lake, in Varese province, Northern Italy. The area is characterized by cold winters when the temperature often reaches 0 °C, and dry and quite hot summers (the temperature reaches 25 °C). Therefore, the farming system had a prevalence of indoor housing in winter and free grazing on alpine pastures from spring to autumn, with indoor housing only during the night (or day in the hottest months). Thus, goats were exposed to natural photoperiod. During winter, the diet was based on *ad libitum* hay with increasing supplementation of concentrate from the dry period to early lactation (from 300 to 600 g/day). The birth season of the studied goats occurred from the 11th of January to the 21st of March. After kidding, goats were milked twice a day until September. From March to November, the goat’s diet was based on fresh forages on pastures (about 200 hectares) between 900 and 1550 m a.s.l. The main vegetal species eaten by goats in this context have been already described (Maggioni, Mondellini, and Corti 2004). Anthelmintic treatment with netobimin 15 mg/kg was administered to all goats in November of the year before the study.

A clinical examination was performed on every goat before being included in the trial, and only apparently healthy subjects were submitted for blood collection (Camosciata delle Alpi, *n* = 37; Verzaschese, *n* = 34). The mean Body Condition Score (BCS) was 2.8/5 in Camosciata delle Alpi and 2.7/5 in the Verzaschese breed. Mean and min–max age for Camosciata delle Alpi goats were 5 (2–8) years, while for Verzaschese goats were 5 (3–12) years. Only pregnant nulliparous, primiparous, and pluriparous goats were included in the study. The mean rectal temperature varied in the range 38.5–39.7 °C. Goats that for any reason at the end of the experimental trial had less than 11 blood samples, were excluded from the study (Camosciata delle Alpi, *n* = 3; Verzaschese, *n* = 2). On the same day of blood collection, milk samples from each goat were collected and analyzed for basic lactation variables (milk yield, fat percentage, protein percentage, lactose percentage, and somatic cell count). The data derived from the milk analysis are reported and discussed in previous work by Agradi et al. (Agradi et al. 2021). Moreover, data about the parasitic load of the same goats included in our study and during the same period analyzed, are discussed in another study by Zanzani et al. (Zanzani et al. 2020).

Collection and Analysis of Blood Samples

Blood samples were collected monthly from every goat included in the study (Camosciata delle Alpi, *n* = 37; Verzaschese, *n* = 34) in the morning before feeding on the same day of the month from January to December by jugular venipuncture using 18G disposable needles into 3 mL sterile vacuum tubes containing K3EDTA as anticoagulant. Blood samples were refrigerated at 4 °C until analysis, which was performed within 8 hours from collection. On whole blood, the following parameters were determined: red blood cells (RBC), hemoglobin (HGB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), leucocyte count (WBC), neutrophil count (NEU), lymphocyte count (LYMPH), monocyte count (MONO), eosinophil count (EOS), basophil count (BAS), neutrophil percentage (NEU fraction), lymphocyte percentage (LYMPH fraction), monocyte percentage (MONO fraction), eosinophil percentage (EOS fraction), and basophil percentage (BAS fraction). N/L (neutrophils to lymphocytes) ratio was calculated as the ratio between neutrophil (NEU) and lymphocyte count (LYMPH).

Hematological analyses were performed in triplicate with the automated apparatus Cell-Dyn3500 (Abbott Laboratories, Abbott Park, IL, USA); veterinary software was implemented in the hematological apparatus.

Data Analysis

To determine the reference intervals, the "Guidelines for the determination of reference intervals in veterinary species" issued by the Quality Assurance and Laboratory Standards committee were followed, as reported by Friedrichs et al. (Friedrichs et al. 2012). First, extreme outliers (first quartiles − 3.0 interquartile range or third quartiles + 3.0 interquartile range) were identified and eliminated (Table S1). Then, the average for each subject and the mean intra-individual coefficient of variation (CV) were calculated for each parameter. The normality of the data distribution of each variable was assessed by a Kolmogorov–Smirnov test (Table S1) with Lilliefors significance correction and histograms. Finally, although most of the parameters showed a normal distribution, a robust method was chosen based on the 5th and 95th sample quantiles to determine reference limits and bootstrap to calculate 90% CI of reference limits. Moreover, mean, median (Mdn), first (Q1) and third (Q3) quartiles, and inter-individual CV were reported.

The data were then analyzed by Mixed Linear Models with diagonal variance structure including month and goat as a repeated measure and as a random factor, respectively. These models evaluated the main effect of breed (2 levels: Camosciata delle Alpi and Verzaschese), season (4 levels: winter, spring, summer, and autumn), and age (as a continuous variable). Sidak corrections were used to obtain pairwise comparisons. The parameter estimates (b) with their standard error were also reported for the age effect. Parity and days in milk were not included in the models because of collinearity with age and season, respectively. Indeed, parity shows an effect on the hematological parameters similar to the one of age as, usually, goats have one birth per year starting from 1 year of age.

Statistical analyses were performed with SPSS Statistics version 25 (IBM, SPSS Inc., Chicago, IL, USA). Statistical significance occurred when $p < 0.05$.

Results

Hematological Parameters' Reference Limits Related to Breed

The descriptive statistics, reference limits, and biological variation for the hematological parameters are presented in Table 1 and Table 2 for Camosciata delle Alpi and Verzaschese goat breeds, respectively. A higher biological variability, mainly intra-individual, can be noticed in the Camosciata delle Alpi than in Verzaschese goats.

Table 1. Descriptive statistics, reference limits, and biological variation for hematological variables in Camosciata delle Alpi goats.

Parameter	Descriptive Statistics				Reference Limits (90% CI)		Biological Variation	
	Q1	Mdn	Q3	Mean	Lower	Upper	Intra-Individual CV (%)	Inter-Individual CV (%)
RBC (M/μL)	12.30	13.13	13.77	12.92	10.40 (9.87–1.41)	14.89 (14.28–5.04)	11	9
HGB (g/dL)	8.21	8.59	9.19	8.61	6.30 (5.96–7.74)	10.55 (9.52–10.84)	11	11
PCV (%)	24.10	24.96	26.62	25.15	20.50 (18.84–23.51)	28.67 (27.43–28.79)	10	8
MCV (fL)	18.77	19.50	20.61	19.67	17.11 (17.02–17.92)	21.87 (21.49–21.94)	7	7
MCH (pg)	6.53	6.64	6.90	6.68	6.30 (6.28–6.42)	7.14 (6.96–7.17)	6	3
MCHC (g/dL)	32.64	34.04	35.33	34.23	31.29 (31.02–31.96)	38.46 (37.22–38.75)	6	6
RDW (%)	30.15	33.06	34.87	32.65	27.10 (26.56–28.58)	36.85 (36.03–36.85)	10	9
WBC (K/μL)	7.48	8.81	9.84	8.66	4.45 (4.23–6.10)	11.97 (10.89–12.36)	29	23
NEU (K/μL)	2.25	3.18	3.84	3.12	1.62 (1.51–1.95)	5.11 (4.19–5.70)	51	32
LYMPH (K/μL)	3.28	4.11	6.13	4.62	2.12 (1.63–2.75)	7.86 (6.76–8.98)	31	37
MONO (K/μL)	0.27	0.37	0.46	0.38	0.21 (0.21–0.24)	0.70 (0.55-0.74)	54	35

Parameter	Descriptive Statistics				Reference Limits (90% CI)		Biological Variation	
	Q1	Mdn	Q3	Mean	Lower	Upper	Intra-Individual CV (%)	Inter-Individual CV (%)
EOS (K/µL)	0.15	0.18	0.29	0.22	0.09 (0.08–0.11)	0.45 (0.34-0.52)	74	46
BAS (K/µL)	0.05	0.07	0.08	0.07	0.02 (0.02–0.04)	0.12 (0.10–0.13)	64	37
NEU fraction (%)	28.38	36.56	44.02	36.91	18.65 (18.06–23.60)	59.83 (48.38–67.22)	36	30
LYMPH fraction (%)	45.88	54.86	61.00	53.47	29.58 (25.84–39.62)	75.74 (67.74–77.76)	24	22
MONO fraction (%)	3.77	4.94	6.11	5.06	2.29 (2.04–3.27)	8.54 (7.16–9.17)	63	33
EOS fraction (%)	1.74	2.69	3.36	2.91	1.08 (0.98–1.35)	6.96 (4.60–7.52)	77	53
BAS fraction (%)	0.60	0.75	1.08	0.86	0.36 (0.34–0.45)	1.56 (1.33–1.67)	66	41
N/L	0.51	0.77	1.03	0.81	0.27 (0.24–0.38)	1.63 (1.29–1.82)	56	47

Q1 = first quartile; Mdn = median; Q3 = third quartile; CV = coefficient of variation; RBC = red blood cells; HGB = hemoglobin; PCV = packed cell volume; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width; WBC = leucocyte count; NEU = neutrophil count; LYMPH = lymphocyte count; MONO = monocyte count; EOS = eosinophil count; BAS = basophil count; NEU fraction = neutrophil percentage; LYMPH fraction = lymphocyte percentage; MONO fraction = monocyte percentage; EOS fraction = eosinophil percentage; BAS fraction = basophil percentage; N/L = neutrophils to lymphocytes ratio.

Table 2. Descriptive statistics, reference limits, and biological variation for hematological variables in Verzaschese goats.

Parameter	Descriptive Statistics				Reference Limits (90% CI)		Biological Variation	
	Q1	Mdn	Q3	Mean	Lower	Upper	Intra-Individual CV (%)	Inter-Individual CV (%)
RBC (M/µL)	14.07	14.42	14.93	14.30	11.99 (11.87–3.08)	15.78 (15.21–5.81)	7	7
HGB (g/dL)	9.31	9.67	10.14	9.61	7.80 (7.51–8.86)	11.03 (10.45–11.23)	7	8
PCV (%)	26.13	27.10	28.21	27.18	23.71 (22.93–25.29)	30.78 (29.69–30.95)	8	7
MCV (fL)	18.25	18.99	19.68	19.09	17.67 (17.57–17.92)	21.54 (20.52–21.75)	7	6
MCH (pg)	6.59	6.70	6.87	6.72	6.36 (6.35–6.46)	7.07 (6.98–7.09)	6	3
MCHC (g/dL)	34.64	35.43	36.91	35.47	32.46 (32.02–33.09)	38.38 (37.45–38.83)	7	5
RDW (%)	32.51	36.05	36.93	34.76	28.63 (28.47–30.22)	38.52 (37.43–38.82)	9	9
WBC (K/µL)	4.93	5.53	7.18	5.90	3.40 (3.19–4.25)	9.15 (7.89–9.54)	27	27
NEU (K/µL)	1.26	1.76	2.11	1.77	0.87 (0.87–1.02)	2.85 (2.45–3.19)	46	33
LYMPH (K/µL)	2.72	3.50	4.35	3.57	1.80 (1.79–2.20)	5.45 (5.12–5.58)	32	31
MONO (K/µL)	0.22	0.28	0.32	0.28	0.17 (0.16–0.18)	0.40 (0.37–0.41)	55	25

Parameter	Descriptive Statistics				Reference Limits (90% CI)		Biological Variation	
	Q1	Mdn	Q3	Mean	Lower	Upper	Intra-Individual CV (%)	Inter-Individual CV (%)
EOS (K/μL)	0.08	0.16	0.21	0.17	0.05 (0.05–0.06)	0.42 (0.26–0.51)	72	60
BAS (K/μL)	0.04	0.05	0.06	0.05	0.03 (0.02–0.03)	0.10 (0.08–0.10)	68	34
NEU fraction (%)	24.18	31.41	35.28	30.13	16.87 (16.73–20.04)	45.85 (37.78–49.30)	34	25
LYMPH fraction (%)	55.55	59.30	67.87	60.42	44.26 (43.21–49.15)	74.65 (71.86–74.81)	18	14
MONO fraction (%)	4.18	4.94	5.65	4.91	2.99 (2.85–3.43)	7.11 (6.13–7.59)	57	23
EOS fraction (%)	1.75	2.68	3.98	2.94	1.12 (1.02–1.35)	6.85 (4.38–9.51)	77	57
BAS fraction (%)	0.68	0.86	1.12	0.93	0.49 (0.49–0.58)	1.61 (1.33–1.70)	63	33
N/L	0.38	0.59	0.71	0.57	0.24 (0.23–0.31)	1.03 (0.85–1.24)	52	40

Q1 = first quartile; Mdn = median; Q3 = third quartile; CV = coefficient of variation; RBC = red blood cells; HGB = hemoglobin; PCV = packed cell volume; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width; WBC = leucocyte count; NEU = neutrophil count; LYMPH = lymphocyte count; MONO = monocyte count; EOS = eosinophil count; BAS = basophil count; NEU fraction = neutrophil percentage; LYMPH fraction = lymphocyte percentage; MONO fraction = monocyte percentage; EOS fraction = eosinophil percentage; BAS fraction = basophil percentage; N/L = neutrophils to lymphocytes ratio.

Effects of Breed, Season, and Age on Hematological Variables

The difference between Verzaschese and Camosciata delle Alpi goat breeds was statistically significant for all the red blood cells parameters (except MCH). Except for the MCV, they were higher in Verzaschese than Camosciata goats. As regards the white blood cells, WBC, NEU (both as count and fraction), LYMPH, MONO, and BAS counts, as well as N/L were higher in Camosciata delle Alpi than Verzaschese. Conversely, LYMPH fraction was higher in Verzaschese than Camosciata delle Alpi (Figure 2 and Figure S1, and Table 3).

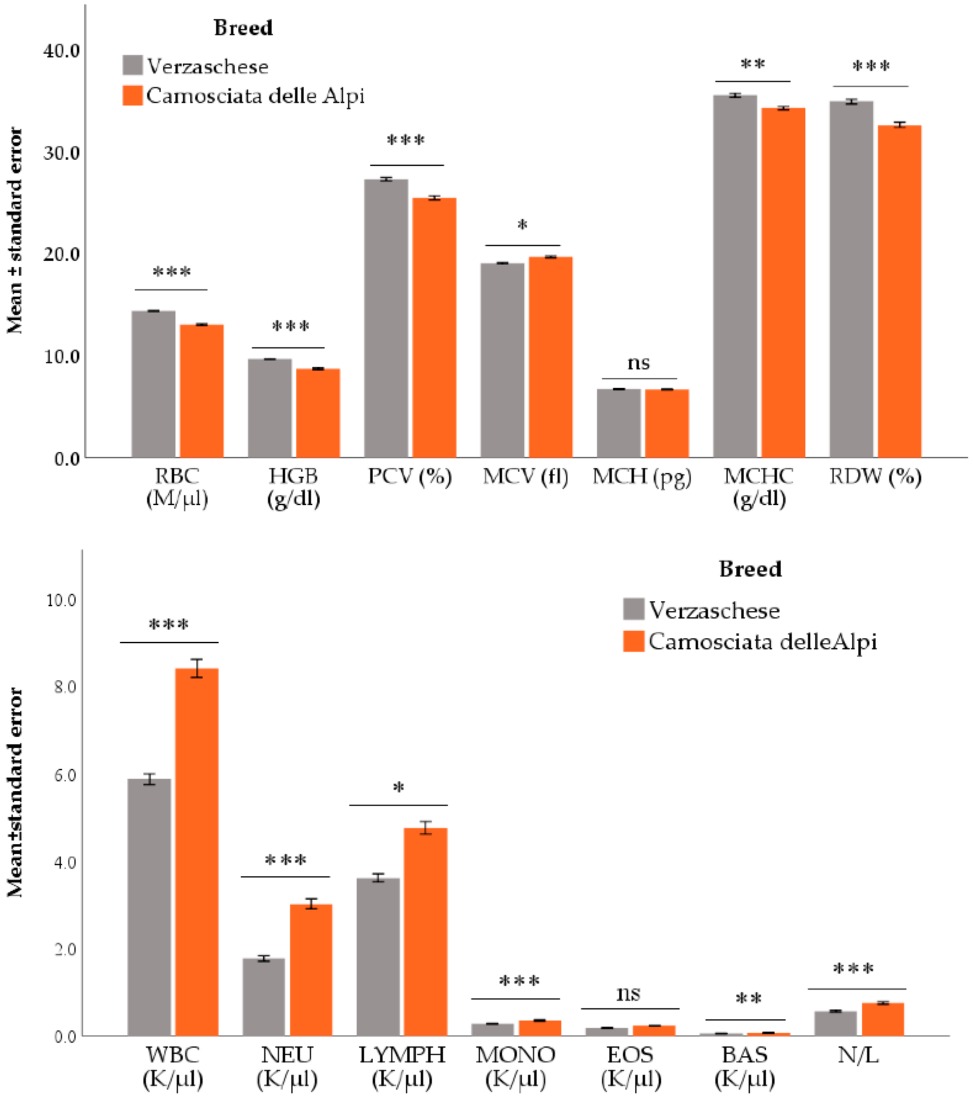

Figure 2. Main effect of breed on the evaluated parameters. Values are means and standard errors. *** p < 0.001, ** p < 0.01, * p < 0.05 Verzaschese vs. Camosciata delle Alpi. Ns = not significant (p < 0.05). Models also included Season and Age (as covariate). RBC = red blood cells; HGB = hemoglobin; PCV = packed cell volume; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width; WBC = leucocyte count; NEU = neutrophil count; LYMPH = lymphocyte count; MONO = monocyte count; EOS = eosinophil count; BAS = basophil count; N/L = neutrophils to lymphocytes ratio.

Table 3. Results of the mixed linear model that analyzed the main effects of breed, season, and age on the evaluated parameters. For the age effect (included in the models as a continuous variable), the parameter estimates (b) with its standard error are also reported.

Variable	Breed Effect	Season Effect	Age Effect	
	p-Value	*p*-Value	b ± Standard Error	*p*-Value
RBC (M/μL)	<0.001	<0.001	−0.24 ± 0.05	<0.001

Variable	**Breed Effect**	**Season Effect**	**Age Effect**	
	***p*-Value**	***p*-Value**	**b ± Standard Error**	***p*-Value**
HGB (g/dL)	<0.001	<0.001	−0.17 ± 0.04	<0.001
PCV (%)	<0.001	<0.001	−0.23 ± 0.12	0.058
MCV (fL)	0.024	<0.001	0.20 ± 0.07	0.004
MCH (pg)	0.549	<0.001	−0.01 ± 0.01	0.878
MCHC (g/dL)	0.001	<0.001	−0.35 ± 0.09	<0.001
RDW (%)	<0.001	<0.001	−0.64 ± 0.14	<0.001
WBC (K/μL)	<0.001	<0.001	−0.35 ± 0.08	<0.001
NEU (K/μL)	<0.001	<0.001	0.04 ± 0.04	0.376
LYMPH (K/μL)	0.049	<0.001	−0.40 ± 0.07	<0.001
MONO (K/μL)	<0.001	0.170	−0.01 ± 0.01	0.411
EOS (K/μL)	0.096	<0.001	−0.01±0.01	0.107
BAS (K/μL)	0.001	0.008	−0.01 ± 0.01	0.969
NEU fraction (%)	<0.001	<0.001	2.13 ± 0.44	<0.001
LYMPH fraction (%)	<0.001	<0.001	−2.34 ± 0.50	<0.001
MONO fraction (%)	0.300	<0.001	0.13 ± 0.07	0.093
EOS fraction (%)	0.987	<0.001	0.02 ± 0.08	0.779
BAS fraction (%)	0.855	0.005	0.04 ± 0.17	0.014
N/L	<0.001	<0.001	0.06 ± 0.01	<0.001

RBC = red blood cells; HGB = hemoglobin; PCV = packed cell volume; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width; WBC = leucocyte count; NEU = neutrophil count; LYMPH = lymphocyte count; MONO = monocyte count; EOS = eosinophil count; BAS = basophil count; NEU fraction = neutrophil percentage; LYMPH fraction = lymphocyte percentage; MONO fraction = monocyte percentage; EOS fraction = eosinophil percentage; BAS fraction = basophil percentage; N/L = neutrophils to lymphocytes ratio.

Seasonal variations were also observed (Figure 3 and Figure S2, and Table 3). Most of the primary red blood cell variables (i.e., RBC, HGB, PCV, and RDW) were lower in summer than in winter and spring. MCV, MCH, and MCHC are exceptions as the lowest values were found in autumn or spring. In spring, the lowest values of LYMPH, MONO, EOS, and BAS fractions were also found. Conversely, spring showed the highest values of WBC, NEU (both as count and fraction), and N/L ratio. LYMPH count was also high in winter while EOS count showed a contrary trend (p <0.05), with the highest values in summer and autumn.

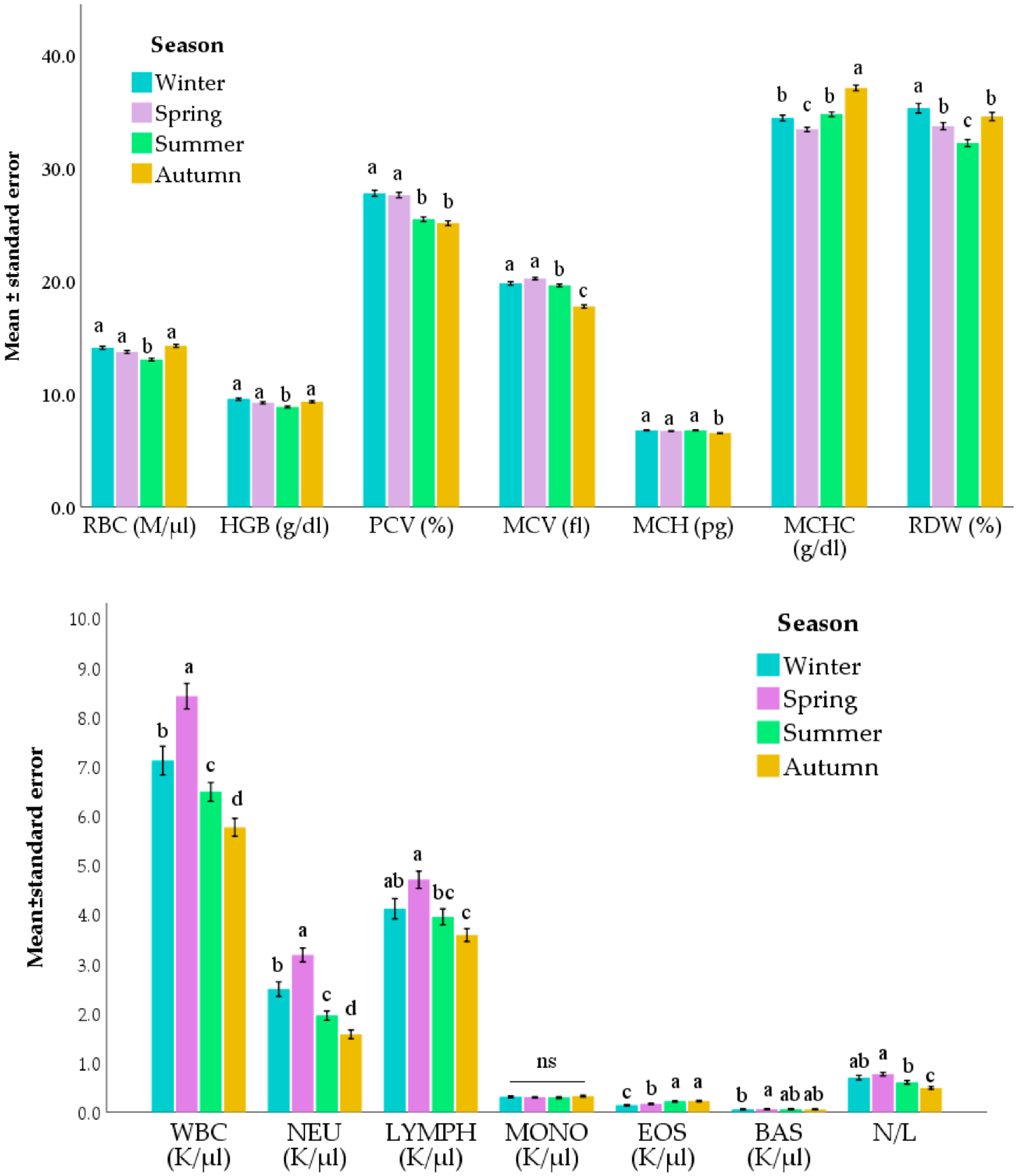

Figure 3. Main effect of season on the evaluated parameters. Values are means and standard errors. For each parameter, bars that do not share the same letter are significantly different (p < 0.05; multiple comparisons with Sidak correction). Models also included Breed and Age (as covariate). RBC = red blood cells; HGB = hemoglobin; PCV = packed cell volume; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width; WBC

= leucocyte count; NEU = neutrophil count; LYMPH = lymphocyte count; MONO = monocyte count; EOS = eosinophil count; BAS = basophil count; N/L = neutrophils to lymphocytes ratio.

The association between age and hematological parameters was defined using the sign of b parameters (Table 3). A positive and significant effect was found for MCV, NEU, and BAS fractions and N/L, while a negative one for RBC, HGB, MCHC, RDW, WBC, and LYMPH (as count and fraction).

Discussion

Hematological Parameters' Reference Limits Related to Breed

This is the first study that has investigated and compared the hematological values in Verzaschese and Camosciata delle Alpi goat breeds. The reference intervals obtained are in general narrower compared to the generic values reported by Schalm et al. (Schalm, Jain, and Carrol 1986), which do not refer to specific breeds but are usually consulted by the practitioners (Jones and Allison 2007; Matthews 2016; Pugh and Baird 2012; Smith and Sherman 2009). That testifies to the higher specificity given by reference limits built for specific breeds rather than for the entire species. In particular, the limits reported by Schalm et al. (Schalm et al. 1986) for some of the red blood cells parameters (i.e., HGB, PCV, MCV, and MCH) resulted to be higher than what was found in Verzaschese and Camosciata delle Alpi goats. The establishment of hematological reference values for different breeds could be of considerable importance in the context of adaptation strategies and, in particular, for the *Capra hircus* which is adapted to many different climates and environmental conditions.

Comparative Hematological Changes Related to Breed, Age, and Season

The examined breeds differed in several hematological parameters, mainly related to the red blood cells. In particular, Verzaschese showed higher mean values for RBC, HGB, PCV, MCHC, and RDW, while MCV was significantly lower than Camosciata delle Alpi goat. These differences could be partly due to subclinical anemia caused by the higher average gastrointestinal parasitic load of Camosciata delle Alpi goats than the Verzaschese goats, as reported by Zanzani et al. in a previous study which investigated the gastrointestinal nematode infections on the same goats and during the same period of our study (Zanzani et al. 2020). Zanzani et al. evaluated the PCV trend along with the strongyle egg output. It was found that the higher strongyle egg output in Camosciata delle Alpi than Verzaschese goat corresponded to a lower PCV. That effect was ascribed to the presence of *H. contortus*, a hematophagus gastrointestinal parasite, whose presence was confirmed by coprocultures on goat feces (Zanzani et al. 2020). Local goat breeds, such as Verzaschese, have shown at a worldwide level a greater resistance and resilience to diseases endemic to their area of origin than cosmopolitan breeds, especially concerning gastrointestinal parasites (Baker et al. 1998; Piedrafita et al. 2010; Zanzani et al. 2019). This can be explained by the fact that the semi-extensive farming system that characterizes the autochthonous breeds could result in a longer co-evolution between pathogen agent and host as well as in greater exposure to harmful organisms than cosmopolitan breeds (Alberti et al. 2014; Piedrafita et al. 2010; Taberlet et al. 2008; Zanzani et al. 2019).

Regarding the leucocyte parameters, N/L ratio is considered a good index of stress, with an increase in N/L ratio proportional to the level of glucocorticoid released (Davis, Maney, and Maerz 2008; Righi et al. 2019). Thus, the lowest N/L which was found in Verzasca goats suggests their greater adaptation to environmental stressors. Interestingly, an unexpected result was that both EOS count and EOS fraction had not shown any significant difference between breeds, notwithstanding the different average gastrointestinal parasitic load between the examined goats of the two breeds (Zanzani et al. 2020).

Age is another factor that could influence the value of some hematological parameters. As found in other breeds (Antunović et al. 2019; Arfuso et al. 2016; Egbe-Nwiyi T, Nwaosu, and Salami 2000; Mbassa and Poulsen 1991; Schalm et al. 1986), RBC, HGB, and MCHC increased with the age. This physiological variation could be due to a greater oxygen-carrying capacity of the younger goats compared with the older ones and, as a consequence, a higher metabolic activity (Daramola et al. 2005). The trend of RDW values was in agreement with previous studies (Smith and Sherman 2009), showing a decline with age. Indeed, poikilocytosis is very common in goat species, especially in young subjects. Conversely, MCV increased with age. That could be a physiological variation to the decreasing trend of many of the erythrocytic parameters although it was in contrast with the study of Antunović et al.(Antunović et al. 2013). Regarding the leukocytic parameters, as shown previously in other goat breeds (Antunović et al. 2019; Arfuso et al. 2016; Tibbo et al. 2004), WBC and LYMPH fractions decreased with age. Moreover, in agreement with the studies mentioned above (Antunović et al. 2019; Arfuso et al. 2016; Tibbo et al. 2004), the NEU fraction increased with age and, as a consequence, N/L ratio had a positive trend. The positive trend of N/L ratio with age has already been demonstrated in human beings (Li et al. 2015). The physiological meaning for this aspect could be probably ascribed to the ability to cope with stressors which becomes dysregulated with aging.

Season too plays a pivotal role in influencing most of the hematological parameters. Several erythrocytic variables, such as RBC, HGB, PCV, and MCV showed lower values in summer (and autumn for PCV, MCV, and MCH). This decrease in the red blood cell parameters could be due to the higher gastrointestinal parasitism which is responsible for causing anemia. Indeed, mixed parasitic infections are common in goat species (Smith and Sherman 2009) and, among others, *H. contortus* was identified as responsible for causing a decrease in the PCV of the goats of this study (Zanzani et al. 2020). Indeed, strongyle egg output in our sampled subjects was previously investigated by Zanzani et al. (Zanzani et al. 2020). It resulted higher during summer and autumn than in other seasons. During these seasons, goats were left free to graze on pastures that were contaminated by eggs and larvae of gastrointestinal parasites due both to the overwintering ability of some parasitic species on grass and to the exit from parasite quiescence in the gastrointestinal tract of others (Taylor, Coop, and Wall 2013). That also explicates the EOS fraction pattern: the lowest values were reported in winter and spring, which are followed by an increase in summer and autumn. Eosinophils are immune cells that assist with the defense against parasites (Smith 2015) and, consistently, they increased during the seasons when a higher strongyle egg output was demonstrated (i.e., summer and autumn (Zanzani et al. 2020)).

Regarding the leukocytic parameters, WBC, NEU, and LYMPH counts had higher values in winter and spring than in summer and autumn. That could be the effect of the melatonin immunostimulating properties. Indeed, melatonin is a hormone with immunomodulatory activity, which has demonstrated positive effects on both innate and acquired immune functions in goats during short-daylength months (Ghosh, Amaresh K. Singh, and Haldar 2014). Goats are short-day breeders and their reproductive activity is strictly linked to the relative duration of the preceding photoperiod, which biologically results in the melatonin release pattern (Alila-Johansson et al. 2001). Todini et al. (Todini et al. 2011) found that in two Italian goat breeds, at latitudes close to those of our study, the plasma melatonin nycthemeral profiles show higher values in winter and spring than in summer, and a significant difference among melatonin concentrations in months with similar day lengths but opposite photoperiods (April and August). In particular, in this investigation, higher concentrations of the hormone were found during spring than at the end of summer (Todini et al. 2011). Thus, we could attribute the significant increment during winter and spring of WBC, NEU, and LYMPH counts to the physiological effect of photoperiod on goats.

Finally, our results regarding the season effect could be used in the evaluation of the days in milk effect because of the collinearity among these two aspects. Considering this, our findings are in general consistent with what is already described in the literature (Antunović et al. 2013; Mbassa and Poulsen 1991). In particular, in agreement with our results, red blood cell parameters showed a negative trend along lactation in the study conducted on Alpine goats by Antunović et al.(Antunović et al. 2013). Regarding white blood cell parameters, our study showed an increase in WBC, NEU, and LYMPH counts during the peri-partum period, and that is consistent with what was observed by Mbassa et al. (Mbassa and Poulsen 1991).

This study has potential limitations especially concerning the “sample bias”. A larger number of subjects and farms and a longer interval of sampling time could improve the external validity of the results. It must be remembered that this would introduce another bias given by the farm management system. The statistical approach was nevertheless robust and followed the indications of the reference interval guidelines (Friedrichs et al. 2012). Moreover, including goats reared under the same management conditions may have reduced the influence on hematological variables of exogenous factors such as nutrition.

Conclusions

In conclusion, our findings confirmed that the hematological reference intervals of the local breed (Verzaschese) differ from that of the cosmopolitan one (Camosciata delle Alpi) and that these differences are indicative of greater resilience towards gastrointestinal parasitism and adaptation to environmental stressors of the local breed, probably as a result of adaptive strategies to the context of breeding developed through centuries. Hematological parameters changed also according to the animal’s age and season. In the latter case, an effect of melatonin could be speculated. The results here presented can add some knowledge to the definition of the health status of the two breeds. Moreover, these findings can be helpful to provide evidence of the importance of recovering endangered/rare local breeds, fundamental heritage for agricultural biodiversity, and local culture. Verzaschese goat represents an agricultural pillar for many regions in Northern Italy and Switzerland, and a breed with unique adaptive characteristics, like other livestock local breeds must be preserved, either by the optimal maintenance of subjects or the conservation of genetics.

Supplementary Materials

Table S1. Significance of the Kolmogorov-Smirnov Tests and numbers of outliers eliminated for each parameter.

Parameter	Sig. Kolmogorov-Smirnov*		Outliers (deleted)
	Camosciata delle Alpi	Verzaschese	
RBC (M/µl)	0.200	0.022	2

HGB (g/dl)	0.200	0.200	0
PCV (%)	0.200	0.200	1
MCV (fl)	0.200	0.200	0
MCH (pg)	0.200	0.200	4
MCHC (g/dl)	0.200	0.200	1
RDW (%)	0.200	0.001	1
WBC (K/µl)	0.200	0.176	0
NEU (K/µl)	0.200	0.200	7
LYMPH (K/µl)	0.100	0.200	1
MONO (K/µl)	0.200	0.200	21
EOS (K/µl)	0.002	0.200	10
BAS (K/µl)	0.200	0.036	7
NEU fraction (%)	0.200	0.200	0
LYMPH fraction (%)	0.200	0.200	0
MONO fraction (%)	0.200*	0.200	20
EOS fraction (%)	0.028	0.200	9
BAS fraction (%)	0.058	0.200	10
N/L	0.200	0.200	9

Null hypothesis: the set of data comes from a Normal distribution.

RBC = red blood cells; HGB = haemoglobin; PCV = packed cell volume; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; RDW = red cell distribution width; WBC = leucocyte count; NEU = neutrophil count; LYMPH = lymphocyte count; MONO = monocyte count; EOS = eosinophil count; BAS = basophil count; NEU fraction = neutrophil percentage; LYMPH fraction = lymphocyte percentage; MONO fraction = monocyte percentage; EOS fraction = eosinophil percentage; BAS fraction = basophil percentage; N/L = neutrophils to lymphocytes ratio.

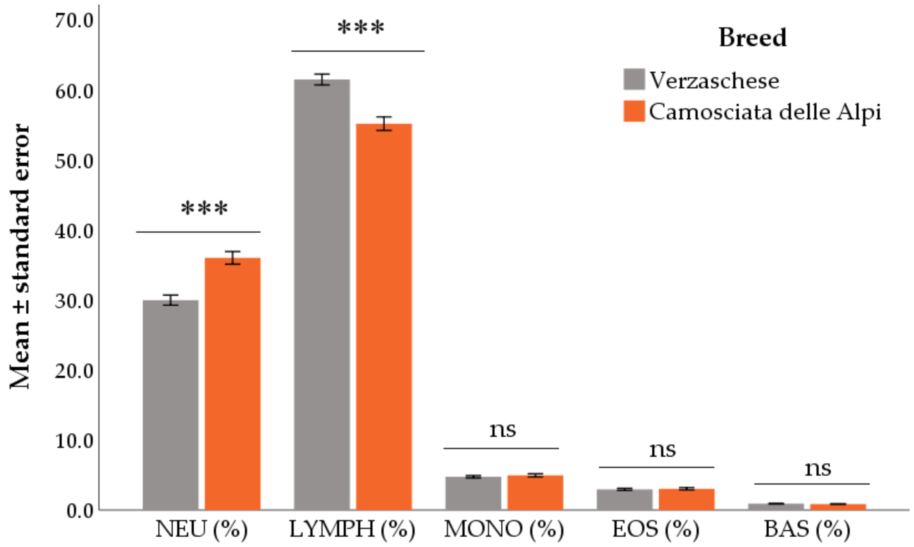

Figure S1. Main effect of breed on the white cells evaluated as fractions. Values are means and standard errors. ***p<0.001 Verzaschese vs Camosciata delle Alpi. ns = not significant ($p < 0.05$). Models also included Season and Age (as covariate). NEU = neutrophil percentage; LYMPH = lymphocyte percentage; MONO = monocyte percentage; EOS = eosinophil percentage; BAS = basophil percentage.

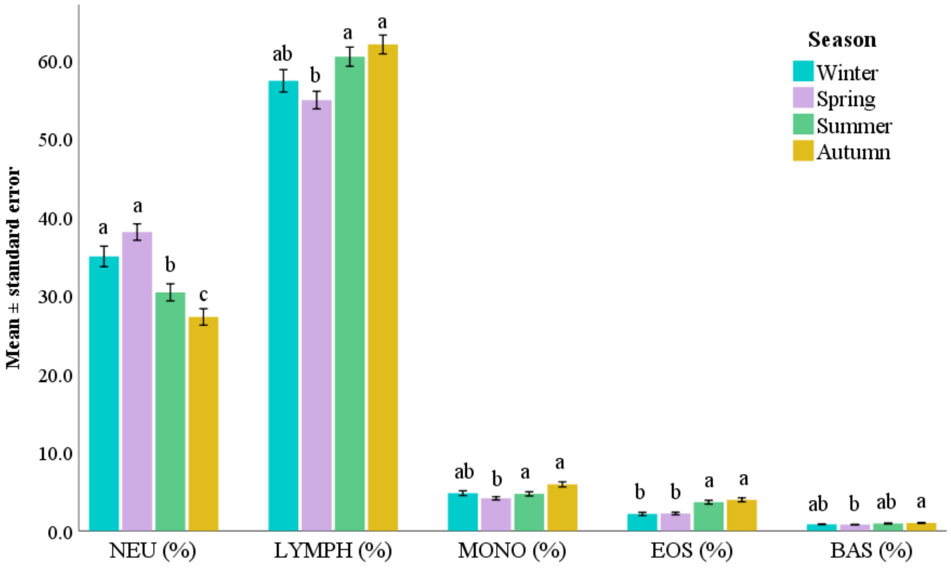

Figure S2. Main effect of season on the white cells evaluated as fractions. Values are means and standard errors. For each parameter, bars that do not share the same letter are significantly different (p<0.05; multiple comparisons with Sidak correction). Models also included Breed and Age (as covariate). NEU = neutrophil percentage; LYMPH = lymphocyte percentage; MONO = monocyte percentage; EOS = eosinophil percentage; BAS = basophil percentage.

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3.5 GOAT HAIR AS A BIOINDICATOR OF ENVIRONMENTAL CONTAMINANTS AND ADRENAL ACTIVATION DURING VERTICAL TRANSHUMANCE

The data shown in this chapter refer to the already accepted paper: Agradi, S., Munga, A., Barbato, O., Palme, R., Tarhan, D., Bilgiç, B., Dokuzeylül, B., Ercan, A.M., Or, M.E., Brecchia, G., Curone, G., Draghi, S., Vigo, D., Marongiu, M.L., González-Cabrera, M., & Menchetti, L. (2023). Goat Hair as a Bioindicator of Environmental Contaminants and Adrenal Activation During Vertical Transhumance. Frontiers in Veterinary Medicine.

Introduction

Since the first livestock animal domestication events around 12,000 years ago, pastoralism has spread all around the world following human migrations (Diamond & Bellwood, 2003). Even in the most complex sedentary farming societies, the need for a continuous source of feed during the year has led to the practice of transhumance. Specifically, for those human populations living near mountainous regions, vertical transhumance still represents a fundamental strategy to guarantee quality grazing through the different seasons thanks to the handling of the flocks and herds along an altitudinal gradient (Makarewicz et al., 2017). That is particularly true when considering the Alpine region of Italy. However, despite the socio-ecological importance of vertical transhumance and its related supply of ecosystem services, that practice is gradually disappearing (Pardini & Nori, 2011).

The maintenance of the traditional semi-extensive farming system of autochthonous goats, sheep, and cattle breeds in Northern Italy, which provides a hay-based diet with indoor housing in winter and fresh forages from late spring to autumn through alpine pastures (Agradi et al., 2020; Battaglini et al., 2014), also plays a useful role in a One-Welfare perspective. Indeed, this system generates a close bond of interdependence between the environment in which these livestock animals are traditionally bred for centuries, if not millennia, and the local breed itself. This leads to the opportunity of using these animals as bioindicators, evidencing harmful changes caused by pollution in the surrounding ecosystem (Parmar et al., 2016), also thanks to the ease with which observations or biological samples can be obtained. Thus, recently, the use of autochthonous livestock breeds to monitor the environmental trace elements and heavy metals levels is growing. These kinds of molecules could be toxic at specific concentrations, and represent a risk for human, animal, and environmental health (Kincaid, 2000; Vardhan et al., 2019). Herbivores are constantly exposed to their uptake *via* ingestion of small amounts of soil, polluted vegetation, and water. It must however be taken into consideration that species-specific physiological characteristics (Hofmann, 1989), as well as seasonal, ecological, and ethological variations, may affect exposition and absorption (Roggeman et al., 2013; Wang et al., 2014). Regarding the human part, the food chain pathway is considered one of the major ways of exposition (Nkwunonwo et al., 2020), and, also for this reason, controlling the possible contamination of products of animal origin, such as cheese or meat, is necessary.

Another aspect that is positively linked to the alpine pasture, is animal welfare. Studies have evidenced that both consumers and farmers consider livestock's access to pasture important, also because of the shared idea that allowing animals to express their natural behaviour is pivotal (von Keyserlingk & Weary, 2017). The European Union encourages these practices through the provision of specific CAP (Common Agricultural Policy) funds. The general aim is to counteract the abandonment of mountain farming areas which, if appropriately used and managed, can contribute to ensure real protection of the territory, biodiversity, prevention of hydrogeological risk and soil erosion, and, consequently, adaptation to climate change (Battaglini et al., 2014; Curone et al., 2019). However, the action of grazing also involves other aspects that affect animals and need to be considered, such as feed quality and availability, predation, parasitosis, environmental conditions, and biosecurity for transmissible diseases between flocks/herds or from wild animals (Aubé et al., 2022). Therefore, animal welfare must be monitored also in grazing animals. A classic welfare indicator is cortisol (Mormède et al., 2007), the end-product of the activation of the hypothalamic-pituitary-adrenal (HPA) axis which is released in response to different types of stimuli (i.e., stressors). The actions of this hormone system are tightly regulated to ensure that the body can respond quickly to the stressors, adjust to these challenges, and recover to the original homeostasis (Mormède et al., 2007; Möstl & Palme, 2002).

A useful tool to evaluate trace elements, heavy metals and cortisol in animals is the analysis of hair. Hair is a metabolically inert biological matrix (after it has left the epidermis) and chemically homogeneous (Perillo et al., 2021). It can be easily collected from domestic animals with a non-invasive modality and, due to its growth pattern, allows for biological retrospective imaging of the element being analysed of even a few months from the time of collection. Finally, the sampling could be repeated on the same skin area, allowing a repeated measure. The analysis of hair enables the determination of both trace elements and heavy metals long-term exposure, thanks to the presence of sulfhydryl group (–SH) of cysteine capable of their chelation (Garcia, 2017). On the other hand, hair cortisol concentration is considered a good indicator of the long-term activation of the HPA axis, used to evaluate animal stress, welfare, and the ability to cope with environmental challenges (Mormède et al., 2007). The use of the hair for the determination of environmental contaminants and animal stress indicators is in perfect agreement with the One-Welfare principles, which invite a holistic consideration of human health and environmental protection in compliance with animal welfare (García Pinillos et al., 2016; Garcia, 2017).

We hypothesized that the content of trace elements and heavy metals as well as of cortisol in the hair of goats changes during transhumance, possibly reflecting different dietary contents and activity of the HPA axis, respectively. Thus, this study aimed to assess the response of an Italian local goat breed to the change from indoor housing to alpine pasture in summer in terms of hair concentrations of (i) trace elements and heavy metals, and (ii) cortisol. Findings could support the possible use of the hair of local breeds as bioindicators of trace elements and heavy metals environmental pollution, and, at the same time, as a non-invasive tool to evaluate the animal's adaptive response to farming system changes in agreement with the One-Welfare principles.

Materials and Methods

Animals and hair collection

For this study, a total of 24 female Frisa goats were enrolled. The Frisa or Frontalasca goat is an autochthonous Lombard goat breed native to the Rezzalo Valley (in the Frontale municipality). The Anagraphic Registry was activated in 1997, and at the end of 2016, the registered population was 785 heads in 51 farms (ASSONAPA Associazione Nazionale Della Pastorizia, 2023; Garcia, 2017). It is a double aptitude breed raised with the alpine traditional farming system. The animals were all part of the same flock, which is bred in Val Bregaglia in the alpine area of Lombardy, near the Swiss border, one of the typical and traditional breeding areas. The animals were kept under a traditional semi-extensive farming system, with indoor housing and a hay-based diet at about 650 m.s.l. from November to May (the hay was purchased from Pavia province, South of Lombardy), and free grazing on alpine pasture at about 1400-2000 m.s.l. in the remaining part of the year (Figure S1 and S2). The reproduction was by natural mating, and the birth season took place in the last two decades of December. For this reason, the goats enrolled were all at the same lactational and reproductive stage (lactating and not pregnant). The goats were milked twice a day, with a milking machine during the indoor housing and by hand during the alpine pasture period, till the second week of August. The milk was entirely used for cheese production. Before including animals in the study, an anamnestic investigation on the sanitary status of the flock was conducted with the breeder, and a physical examination of all goats was conducted by a team of veterinarians. Only clinically healthy animals were enrolled. The mean Body Condition Score was 2.7/5 at the beginning of the study. Nulliparous and primiparous goats were not enrolled in the study. The mean (and standard deviation) age was 6.2±2.9 years (range: 2.2-12.3 years). The involved animals were sampled for 2 consecutive years (2021 (n=10) and 2022 (n=17) for trace elements and heavy metals and 2021 (n=6) and 2022 (n=17) for cortisol) according to the same experimental plan and, consequentially, during the same year period. The experimental design was repeated in the same conditions (same farm, same transhumance, same pastures). The collection of hair samples from live animals was performed in respect of animal welfare according to current legislation. The study was conducted with the approval of the Institutional Animal Care and Use Committee of Università degli Studi di Milano (Permission OPBA_04_2021). Hair samples were collected from the left rump of each animal in a region of about 10 cm2 clipped as close to the skin as possible with an electric clipper, according to the "shave-reshave" method, and stored in polyethylene bags at room temperature protected from sunlight till analysis (Endo et al., 2018; García Pinillos et al., 2016). Hair was shaved the first time one month before vertical transhumance. Then, regrown hair was taken from the same area one day before vertical transhumance (before grazing), one month and two months after the vertical transhumance. Only regrown hair was analysed to determine trace elements, heavy metals and cortisol concentrations, because only for this kind of sample the re-growth time was known (30 days). The present protocol is described in detail by Heimbürge et al. (ASSONAPA Associazione Nazionale Della Pastorizia, 2023; Heimbürge et al., 2019).

Hair analysis

Chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), zinc (Zn), arsenic (As), boron (B), nickel (Ni), lead (Pb), and aluminum (Al) hair concentrations were determined by an inductively coupled plasma-optical emission spectrophotometer (ICP-OES; Thermo iCAP 6000series) at Trace and Toxic Element Analysis Laboratory at Biophysics Department, Faculty of Medicine, Istanbul University-Cerrahpasa (device parameters are reported in Table S1, wavelengths used to determine each element are reported in Table S2). One thousand ppm of each element analysed (Chem-Lab NV, Zedelgem, Belgium) were used to prepare the standard solutions in deionized water. The blank solution was distilled water. These solutions were used to obtain reproducible and linear calibration curves and calculate the correlation coefficient. The analysis was conducted in triplicate. Wet decomposition method for trace elements and heavy metals measurements was used to prepare all samples, using 0.04 g per sample. The hair was added with 2 ml 65% HNO3 (Merck, Darmstadt, Germany), 1 ml 60% HClO4 (Panreac, Barcellona, Spain) and dissolved in a drying oven (Heraeus W.C., Hanau, Germany) at 180 °C. Then, the samples were let to cool down at room temperature and vortexed after adding distilled water until a volume of 10 ml was reached. The element's concentration was determined considering the weight of every single sample and expressed as µg/g wet weight of samples.

For hair cortisol analysis, 100 mg (0.100±0.0005g) of each hair sample were mixed with 5 ml of methanol (100%) and shaken on a thermomixer (37°C for 24 h). Then, the samples were centrifuged (15 min at 2.500 g) and 2.5 ml of the solution were transferred in another tube and dried down (50-60°C under a stream of air). Afterward, steroids were re-dissolved in 0.5 ml of enzyme immunoassay (EIA) buffer and shaken for 30 min. The solution was transferred into tubes (Bio-Rad Laboratories, Hercules, USA) and stored at -20°C until analysis. Twenty-five µl of the stored samples were analysed (in duplicate) in a cortisol EIA. This EIA was performed on microtiter plates and utilizes an antibody raised in rabbits against cortisol-3-CMO:BSA and a biotinylated label. Details of the EIA (including cross-reactions of the antibody) can be found in Palme & Möstl (Palme & Möstl, 1997). The assay was already successfully applied for measuring hair cortisol in ruminants (Salaberger et al., 2016; Stubsjøen et al., 2015).

Statistical analysis

Data were presented as means and standard errors. Diagnostic graphs and Kolmogorov-Smirnov tests were used to verify assumptions. Ni, Pb, Al, Fe, and cortisol were log10(x+1) transformed but the raw data was represented in tables and figures. Changes over time were analysed using Linear Mixed models. Year (2 levels: 2021 and 2022) and Time (3 levels: before grazing, after 1 month, and 2 months of grazing) were included in the models as repeated measures while animals as a random factor. A diagonal structure was chosen as the covariance matrix. Preliminary analyzes had shown that there were no significant influences of the Year or of the Year x Time interaction (Figure S3). Thus, in accordance with the aim of the study, only the Time (i.e., changes during transhumance) was finally included in the models as main effect. The Sidak method was used for multiple comparisons. Statistical analyses were performed with SPSS 25.0 (SPSS Inc. Chicago, USA) and considered as significant at a level of 0.05.

Results

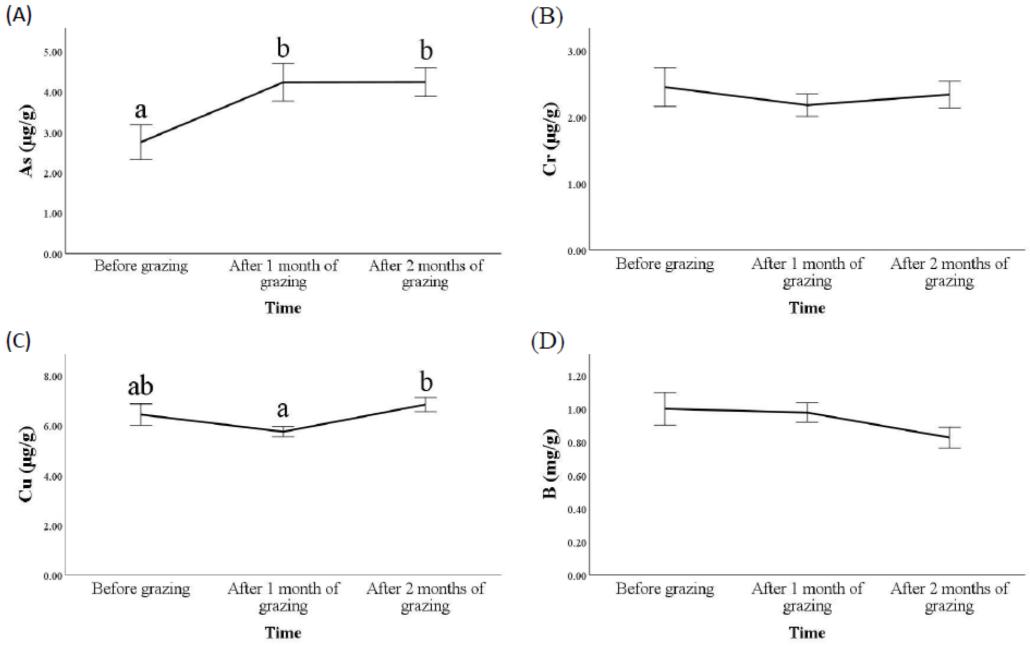
Trace elements and heavy metals

Significant changes over time were found for As (*p*=0.006), Cu (*p*=0.002), Mg (*p*=0.002), Zn (*p*=0.004), and Al (*p*=0.001; Figure 1).

An increase after 1 month of grazing was found for As followed by a stabilization in the second month (*p*<0.05). Cu showed instead a u-shaped trend where the increase was significant after two months of grazing compared to the previous month (*p*=0.003), but the last time point did not differ from the pre-grazing values (*p*=0.778).

Conversely, Mg and Zn showed a progressive decrease with significant differences compared to the indoor values only after two months of grazing (*p*<0.05). Al showed highly variable values after 1 month of grazing but still a reduction after two months compared to pre-grazing (*p*=0.012).

No significative differences among time points were found for the other heavy metals and trace elements concentrations analysed.



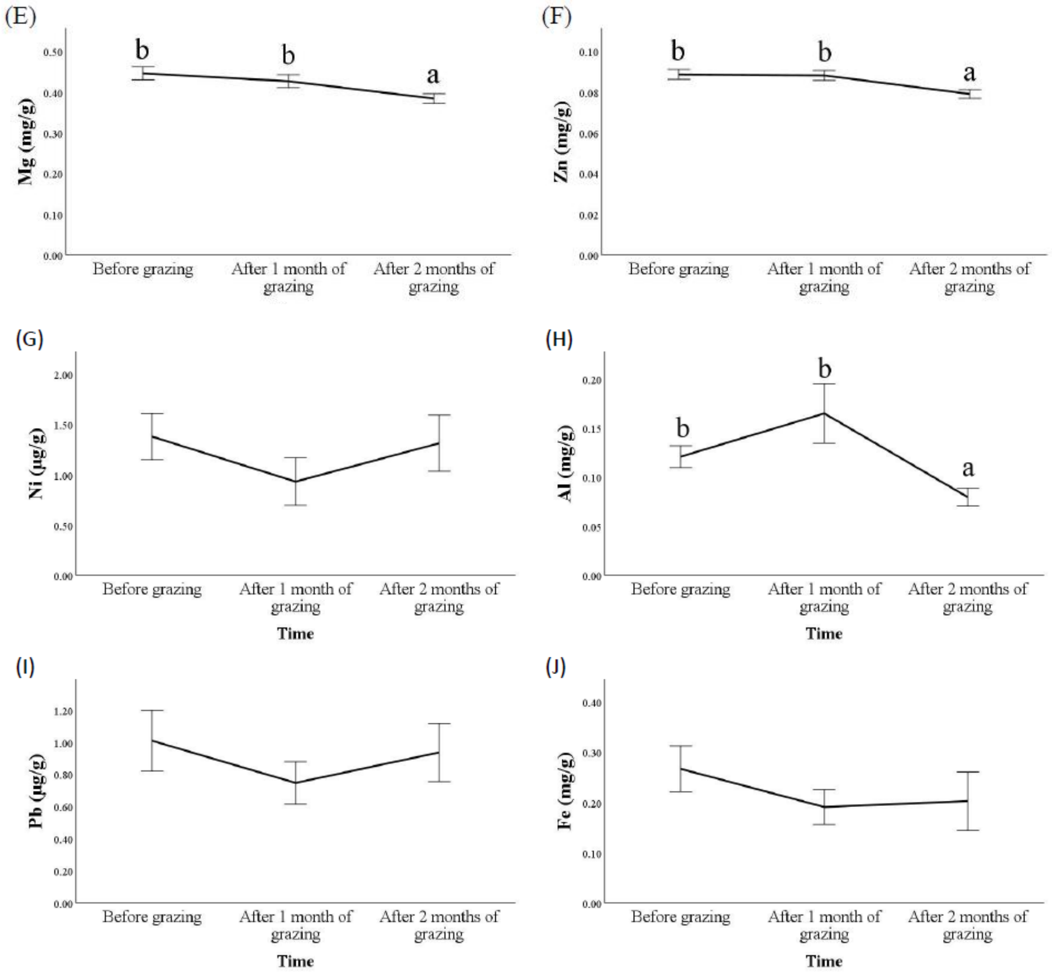


Figure 1. Trace elements and heavy metals' hair concentrations, expressed as µg/g or mg/g of hair, of Frisa goats during vertical transhumance. Hair samples were collected for two consecutive years (n=24 during 2021 and n=17 during 2022) one day before vertical transhumance (before grazing), one month, and two months after vertical transhumance. For each molecule, time points that do not share the same letter are different for *p*<0.05. The time effect was not significant for molecules where no letters are present. The figures show the results for As (A), Cr (B), Cu (C), B (D), Mg (E), Zn (F), Ni (G), Al (H), Pb (I), and Fe (J).

Cortisol

As regards the hair cortisol concentrations (Figure 2), a progressive increase was found in the animals after placing them in the pasture (*p*<0.001). Its values increased already after one month of pasture (2.34±0.41 ng/g) compared to those kept indoors (1.18±0.18 ng/g; *p*<0.001) and remained higher even after two months of grazing (2.75±0.58 ng/g; *p*=0.004). During grazing, an increase in variability can also be noted.

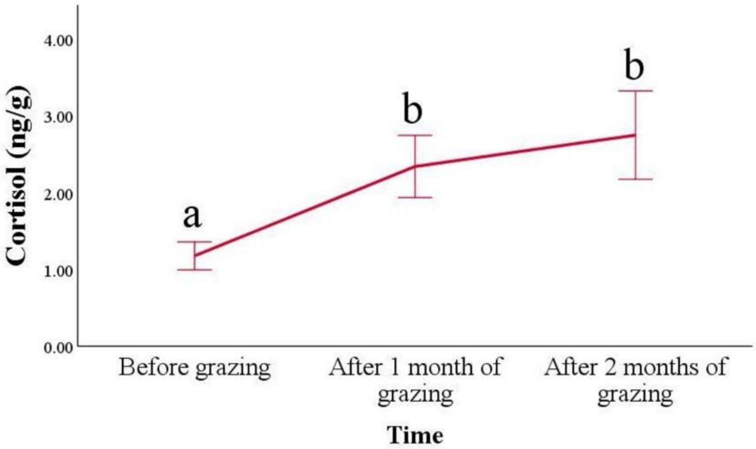


Figure 2. Hair cortisol concentrations (mean ± SE) of Frisa goats during vertical transhumance. Hair samples were collected for two consecutive years (n=6 animals during 2021 and n=17 animals during 2022) one day before vertical transhumance (before grazing), one month, and two months after vertical transhumance. Time points that do not share the same letter are different for $p<0.05$.

Discussion

This study supports the use of the goats' hair as a bioindicator of environmental pollution as its content of some heavy metals and trace elements changed during vertical transhumance in agreement with the change of environment and diet. Findings also showed an increase in hair cortisol concentrations suggesting an activation of the HPA axis linked to the change in the farming system.

Regarding the first aim of our investigation, the results showed an increase in As hair concentration already after 1 month of grazing, which was also maintained during the second month. On the other hand, Mg, Zn, and Al concentrations decreased after 2 months of alpine pasture. The appropriateness of using livestock animals to monitor the heavy metals and trace elements concentrations in the environment has been demonstrated with different biological matrixes, including blood (Fazio et al., 2022; Giannetto et al., 2022; Pavlata et al., 2011), liver and kidney (Mundała et al., 2016), and hair (Fazio et al., 2022; Giannetto et al., 2022; Rashed & Soltan, 2005). To date, the investigations have covered various animal species. In particular, numerous studies have been conducted on wild animals (Draghi et al., 2023) while, among domesticated animals, most of the studies enrolled cows and sheep (Miedico et al., 2016; Or et al., 2005; Wang et al., 2014). Conversely, although the use of goat hair to monitor environmental contamination with heavy metals and trace elements has been validated (Stubsjøen et al., 2015), the literature on this species is scarce. For this reason, there are no reference intervals established for heavy metals and trace element concentrations in goat hair. However, the values obtained in the present study for Zn, Ni, Pb, and Fe are in line with the available reports (Hashem et al., 2017; Mundała et al., 2016; Pavlata et al., 2011; Rashed & Soltan, 2005) while several authors found lower values for As (Biswas et al., 2000; Hashem et al., 2017; Patra et al., 2013; Rana et al., 2012). It is important to underline that, in any case, the contents of As are much lower than those obtained in experimentally or naturally induced chronic arsenicosis (Biswas et al., 2000; Patra et al., 2013; Rana et al., 2012). The explanation for this result lies in the specific As spatial distribution in Italy. Indeed, the Italian Alps are one of the most As enriched areas of the peninsula, due to their pedogeochemical characteristics. Specifically, Val Bregaglia, which is the breeding area of the goats enrolled in our study, shows As anomalies in agricultural and grazing land soils, and in the stream water, which represent the feed sources of the enrolled animals (Zuzolo et al., 2020). Moreover, considering the high affinity of inorganic As compounds to bind to sulfhydryl groups and the amount of cysteine in the hair structure (Shen et al., 2013), the high concentration of As in the hair of Frisa goats could be easily explained. The incremental trend in As concentration during alpine pasture is also attributable to the different distribution of As in soil and water in the Lombardy region. In fact, the hay fed to the animals during the period of indoor housing came from Pavia, a province in the Po River plain where the alluvial sediments are also enriched in As, but at a lower level with respect to the Alpine arch soil, as reported by the EuroGeoSurveys GEMAS (Geochemical Mapping of Agricultural and Grazing land Soil) project (Zuzolo et al., 2020). Regarding Mg, to our knowledge, there are no other studies on goats investigating its concentration in hair. Its values showed a significant decrease after 2 months of alpine pasture. In a study conducted in Switzerland evaluating the spatial distribution of the plant-available forms of Mg in soil, it has been demonstrated that in general, permanent grasslands had a higher amount of available Mg forms than the alpine pastures. That difference has been ascribed to the fertilization with organic manure, which is a common practice in the maintenance of permanent grasslands, and which enriches the soil with various elements, including Mg (Frau et al., 2020). The same difference in land use is present in our study, where the hay fed before vertical transhumance is produced in the permanent grasslands of the Po River valley, while during summer grazing, the only source of feed is the alpine pasture. Regarding Zn, the values obtained in our study were consistent with the scientific literature (Hashem et al., 2017; Pavlata et al., 2011). Its temporal pattern was the same as Mg. In that case, as reported by another investigation on indoor and alpine pasture cow cheeses, the variability of Zn content in biological matrixes of animals is subjected to several factors (Manzi et al., 2021), including the soil composition (Cicchella et al., 2015), the mineral content of the alpine pasture plants (Garmo et al., 1986), and feeding behaviour of the animals (Hofmann, 1989). For this reason, it is difficult to point out a single explanation for its fluctuations. Finally, Al hair concentration follows the same time trend as Mg and Zn, decreasing after 2 months of alpine pasture. Also in that case, the major route of entrance in the animal organism is the ingestion of contaminated feed. Different anthropogenic activities are responsible for the Al soil enrichment and consequent increase of the bioavailability of this compound in plants (Kisnieriene & Lapeikaite, 2015). In a study on roe deer hair (Draghi et al., 2023), the animals which fed near urban areas had higher hair concentrations of Al than animals feeding in rural areas (Draghi et al., 2023). In the same way, we could hypothesize that the difference in Al values before and after vertical transhumance could be due to the different origins of the feed. Indeed, the hay administered during indoor housing was from Pavia province, which is greatly urbanized if compared to the alpine pasture area where the goats fed after the transhumance. Interestingly, in the case of both Mg, Zn and Al, it took two months to produce any change in the hair of goats, which highlights the necessity of chronic exposure to have detectable changes in the hair matrix. Therefore, for the results obtained from our study, we can confirm that the sampling of hair from autochthonous breeds of livestock could be a valid tool as a bio-indicator of environmental contamination by heavy metals and trace elements, with positive implications for the safeguard of humans and environmental health.

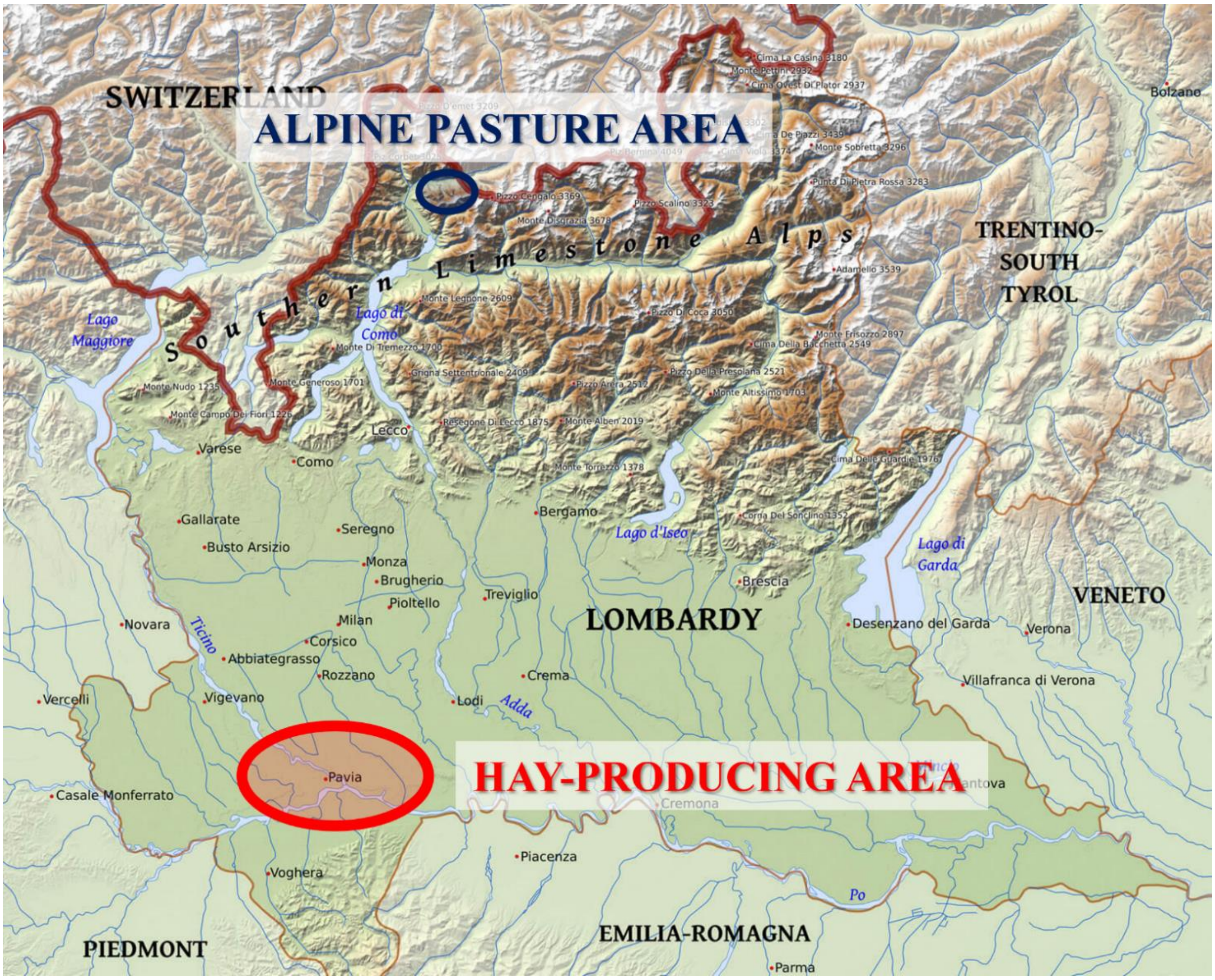
According to the One-Welfare approach, however, the well-being of humans and the environment cannot be disconnected from that of animals (García Pinillos et al., 2016; Garcia, 2017). Thus, in addition to the determination of contaminants, the same, non-stressful, hair sampling was exploited to quantify an important physiological indicator of animal welfare such as cortisol. It also increased the innovativeness of the study because changes in hair cortisol during goat transhumance have not yet been investigated. Results showed a progressive increase in hair cortisol concentration after the vertical transhumance. The values remained high even during the second month of alpine pasture. In goats, the validation of hair cortisol concentrations as an index of long-term activation of the HPA axis was already demonstrated by Endo et al. (Endo et al., 2018). It must be noted that the activation of the axis is not necessarily linked to negative stimuli and has primarily an adaptive function (described by the classic General Adaptation Syndrome, GAS) (Möstl & Palme, 2002). Indeed, also reproductive and physical activities, positive emotional states, and metabolic processes can induce an increase in the production of cortisol by the adrenal gland (Heimbürge et al., 2019). Moreover, non-animal factors including environmental and climatic changes as well as the risk of predation, diseases, and social conflicts may result in HPA axis activation as an adaptive response (Rubenstein & Alcock, 2018). In the present study, several possible biases were eliminated by including only animals of the same sex, age group, physiological status, and coat colour. Therefore, mainly the factors linked to transhumance remain as possible explanations for the cortisol changes. To our knowledge, there are no other studies investigating the variations in cortisol concentration in any biological matrixes of goats moving from indoor to outdoor farming systems. However, Comin et al. evaluated hair cortisol concentration variations in cows moving from valley farms to summer pastures (Comin et al., 2011). They used the same experimental design, and their results are strongly consistent with our findings. Indeed, the hair cortisol concentration increased one month after transhumance and remained constant even 2 months after moving in the cows as well. The values obtained by these authors are in line with those obtained in the present study although cows had higher basal hair cortisol concentrations. We hypothesized that the environmental change during transhumance acts per se as a stressor (intended as a generic stimulus that requires adjustment or coping strategies (American Psychological Association, n.d.; Chu et al., 2022; Selye, 1950)) determining the activation of the GAS and, therefore, of the HPA axis. The maintenance of high concentrations of cortisol can then be due to the increased physical activity in the search for feed sources, which on alpine pastures are spread over several hectares. That enabled the performance of the animal's behavioural repertoire and must therefore be viewed positively in terms of animal welfare. We tend to discard the presence of other specific stressors such as the presence of predators. The wolf (*Canis lupus italicus*) and the bear (both *Ursus arctos marsicanus* and *Ursus arctos arctos*), the only predators in Italy for the adult domestic goat, indeed, were not present in the considered area (Istituto Superiore per la Protezione e la Ricerca Ambientale, 2022; Parco Nazionale Molise d’Abruzzo e Lazio, 2016; Provincia Autonoma di Trento, 2019). Parasitosis also seems implausible as the late spring-summer period, during which the experimental trial took place, is characterised by a lowering trend of the parasitic load on the pasture due to the progressive reduction of humidity and increased UV intensity (Zanzani et al., 2020). Negative interspecific interactions are also likely to be discarded as a cause of the increased cortisol, as the animals did not mix with other flocks during the alpine grazing period and the space available per head increased considerably as a result of the passage to an extensive system. Our findings also showed a higher variability of hair cortisol concentration during grazing, indicating individual differences in HPA axis reactivity and thus subjective responses to the change in the farming system.

Finally, it could be mentioned that the effect of year and its interaction with transhumance were not significant for any variable evaluated. This indicates that there had been no relevant changes in heavy metals and trace elements concentrations in the areas under study and that the effect of transhumance on the HPA axis does not change over the years. Several sources of pollution, such as intensive agriculture and industry, can lead to changes in soil contamination but these are generally evident in the medium to long term (Gil et al., 2018; Yang et al., 2021). Thus, two years of monitoring (as carried out in the present study) may be insufficient to highlight the temporal evolution of trace elements and heavy metal accumulation. We didn't even have a rationale for hypothesizing changes in cortisol concentrations between years. For these reasons, the evaluation of temporal variations was beyond the scope of the present paper. However, a longer observation period, or rather, the systematic sampling of animal hair, might be useful for monitoring the time evolution of contaminants distribution as well as animal welfare.

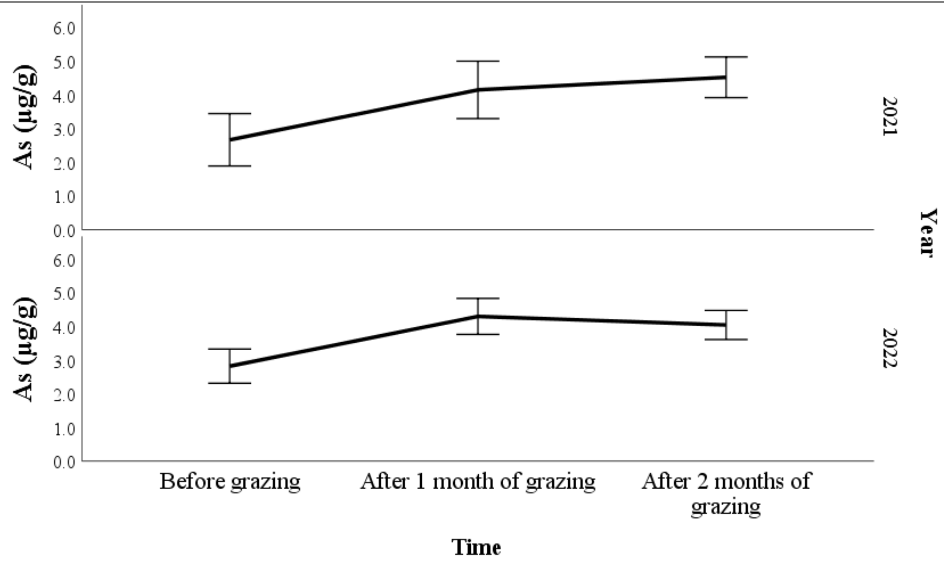
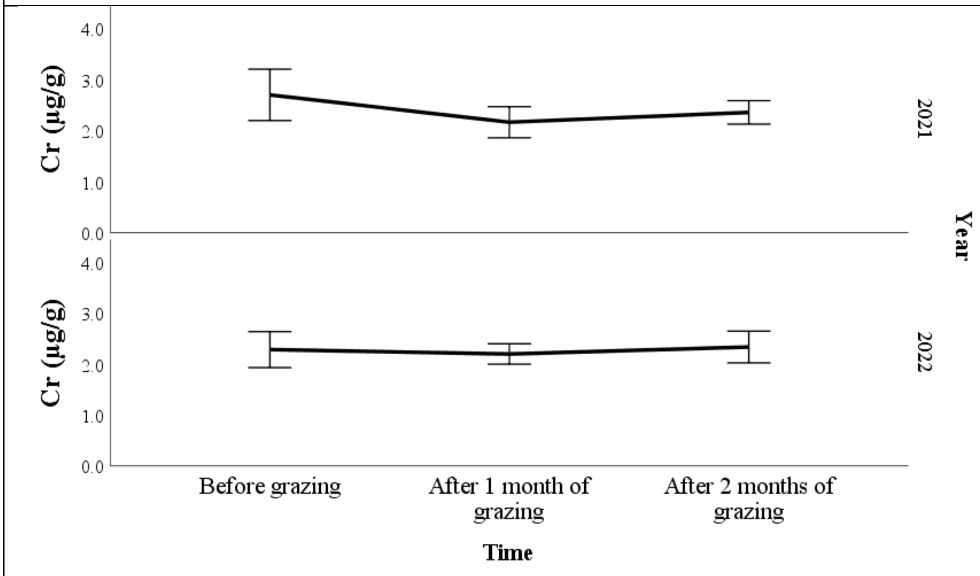
In conclusion, the close bond among autochthonous livestock breeds, such as goats, and the surrounding environment where they live, is a factor to be exploited for studies on environmental changes. The hair is a non-invasive matrix that can also provide information on the activation of the HPA axis and, therefore, on the maintenance of homeostasis and the success of animal adaptation. All this complies with the modern concept of One-Welfare which emphasizes the link between animal welfare, human wellbeing, and the environment. The hair offers practical benefits as well. It is easy to be collected and allows repeated measurements over time on the same subject. In addition, this matrix also gives cumulative results, making it possible to eliminate any daily fluctuations, a factor that is particularly important when assessing stress and well-being, especially in the case of cortisol. We demonstrated the possible associations among environmental and hair trace elements and heavy metals concentrations, specifically regarding As case. Moreover, the hair cortisol concentration allowed for the evaluation of the stress response during vertical transhumance, and we showed an increase in the activation of the HPA axis during the first 2 months of the alpine pasture. Future investigation should include a longer period of evaluation for both the hair trace elements and heavy metals, and hair cortisol concentrations. Moreover, other indicators of stress adaptation and welfare, such as dehydroepiandrosterone, should be evaluated, together with the determination of trace elements and heavy metals also in local soil, water, and plants.

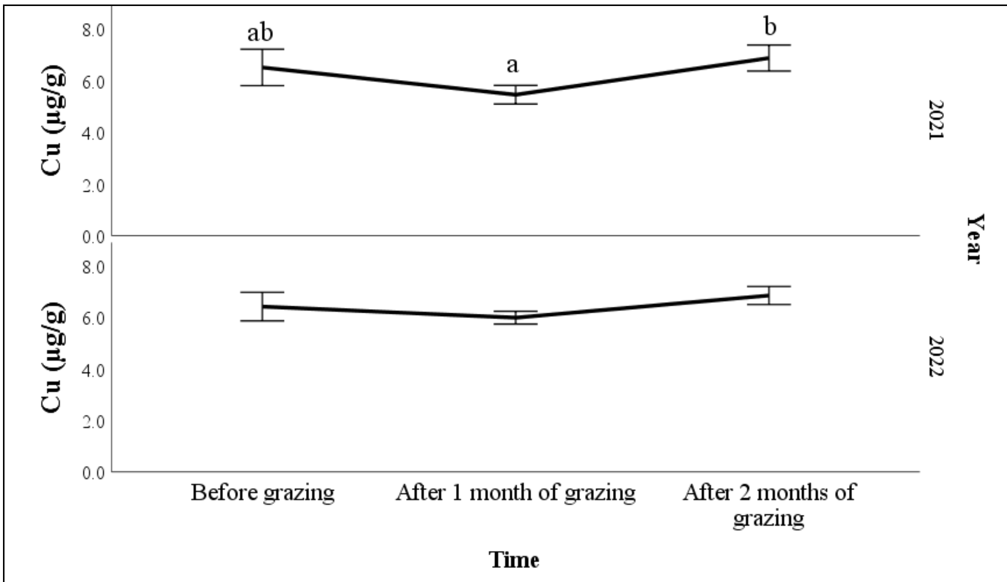
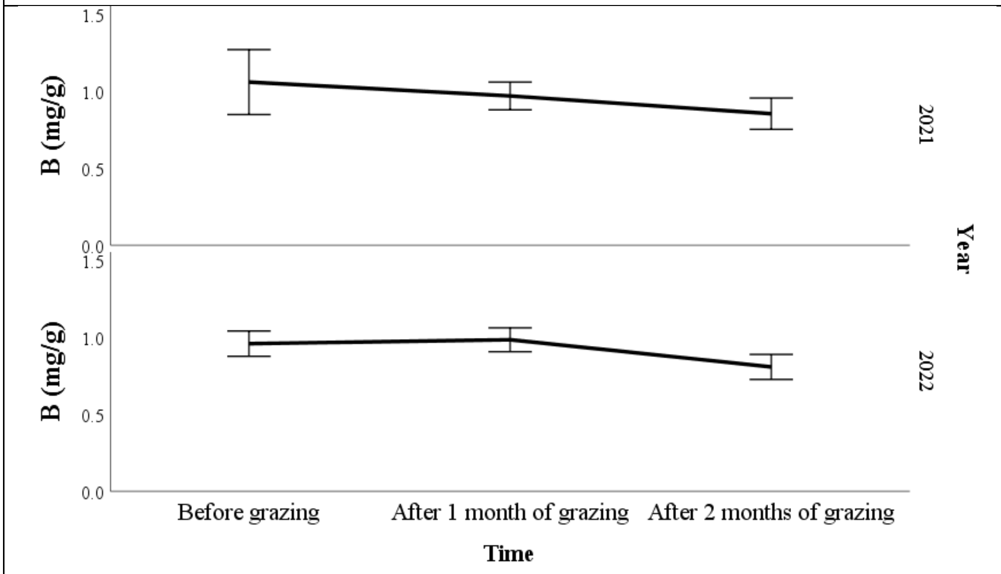
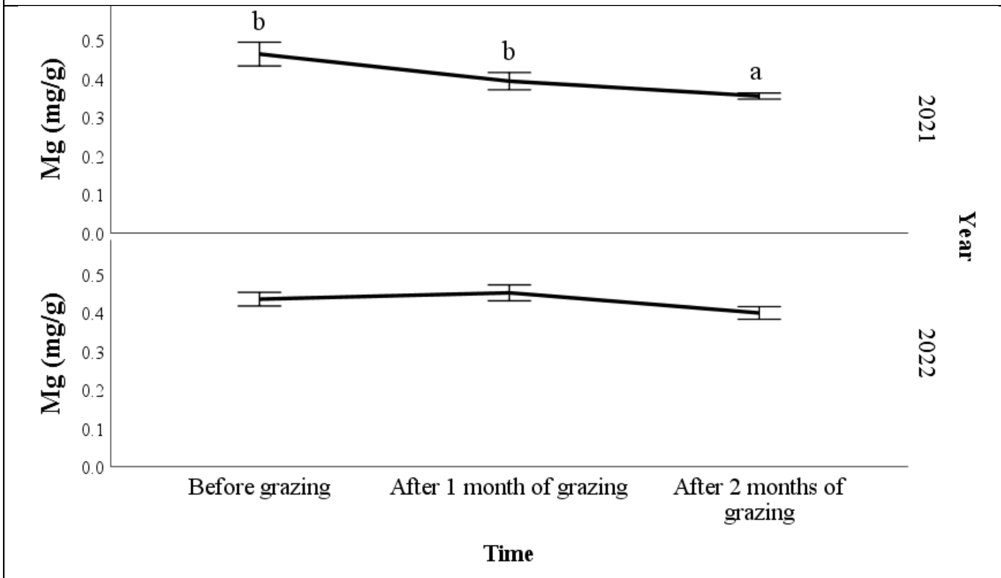
Supplementary Materials

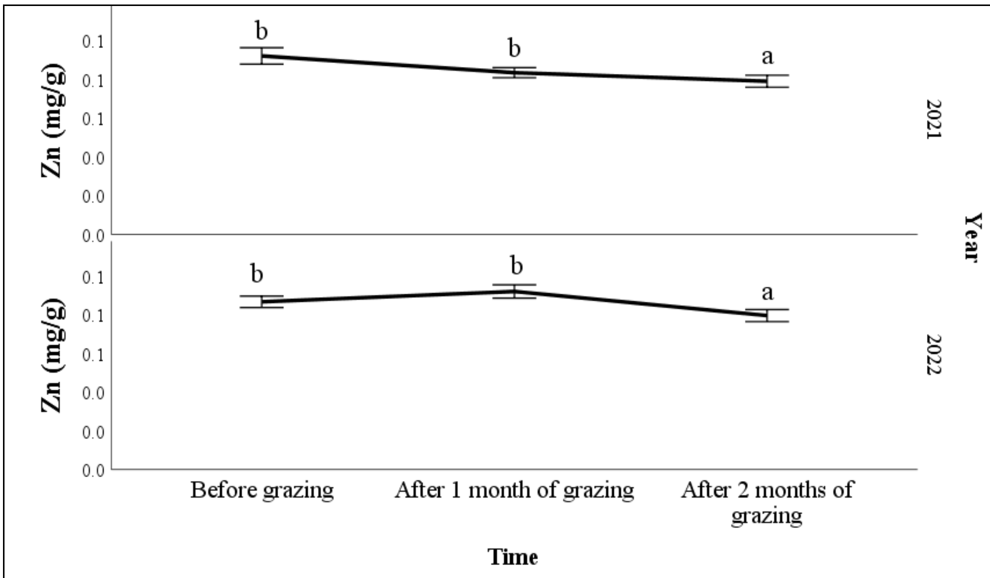
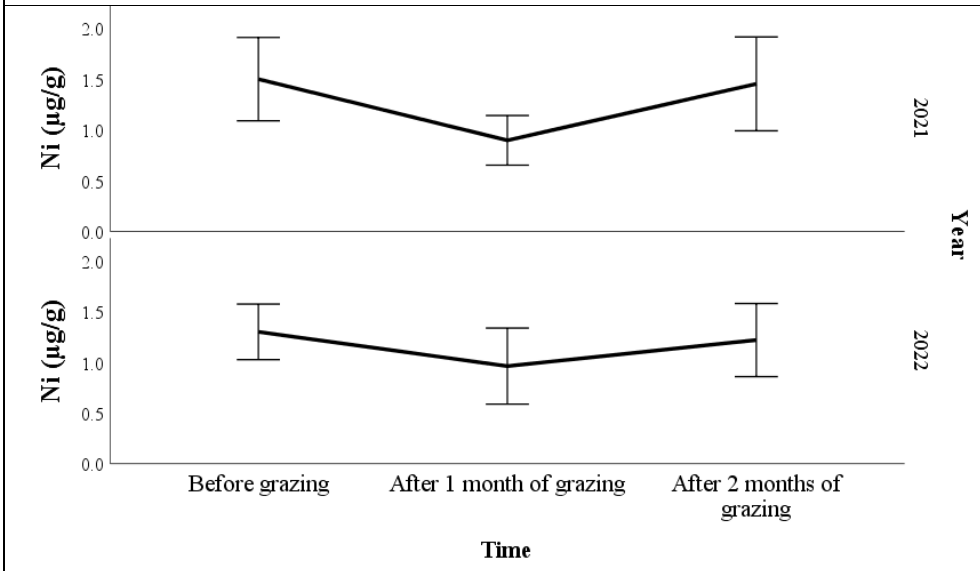
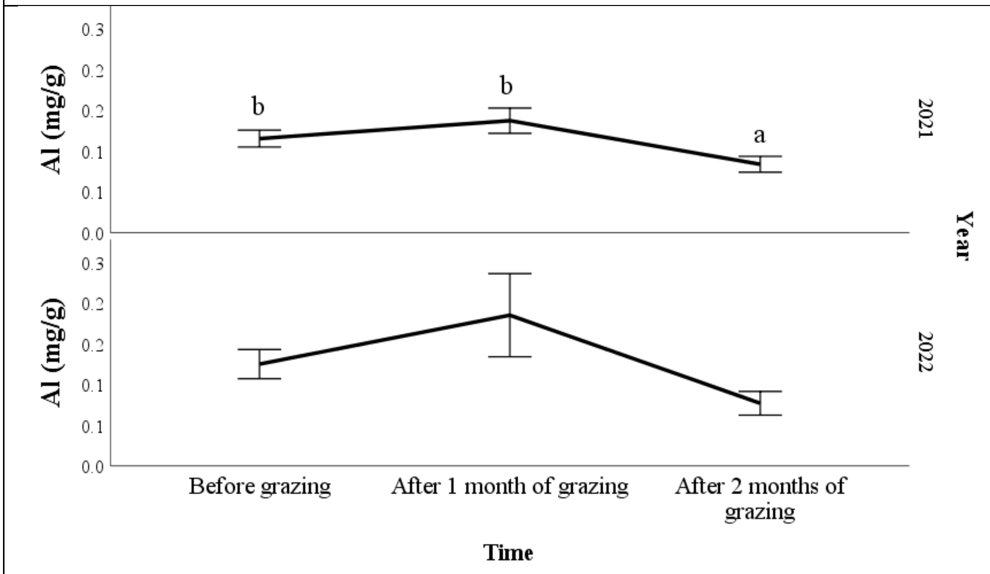
Supplementary Figure 1. Frisa goats grazing on alpine pasture during summer (2021).

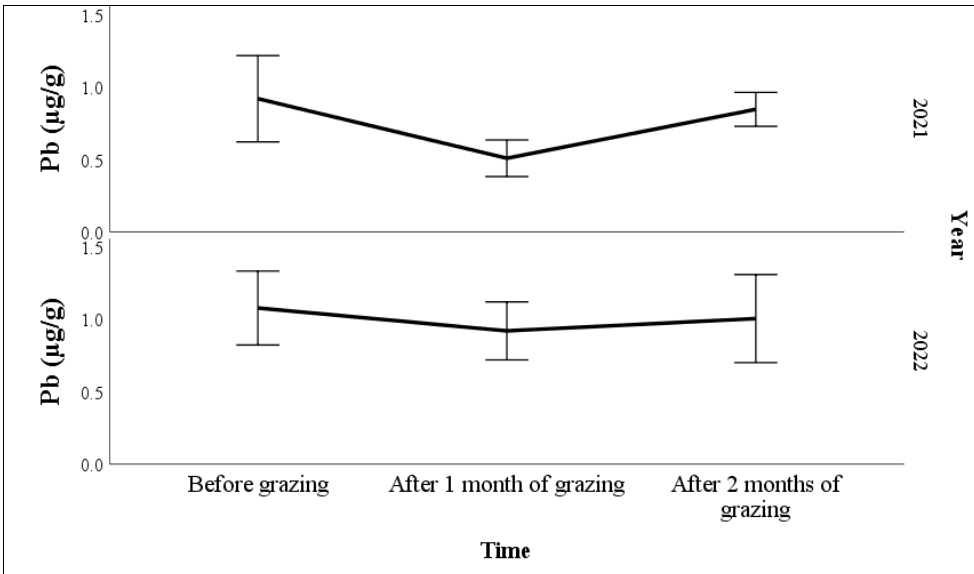
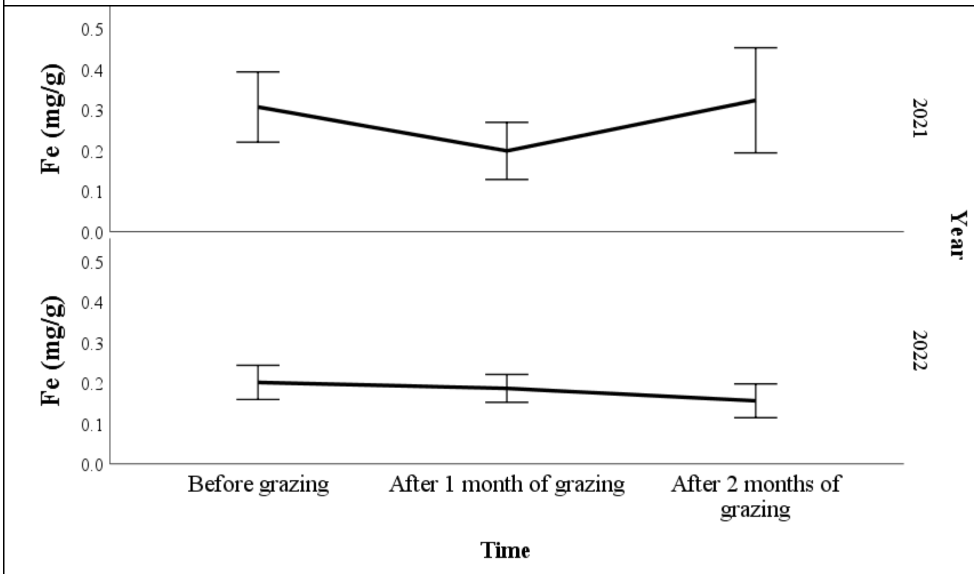
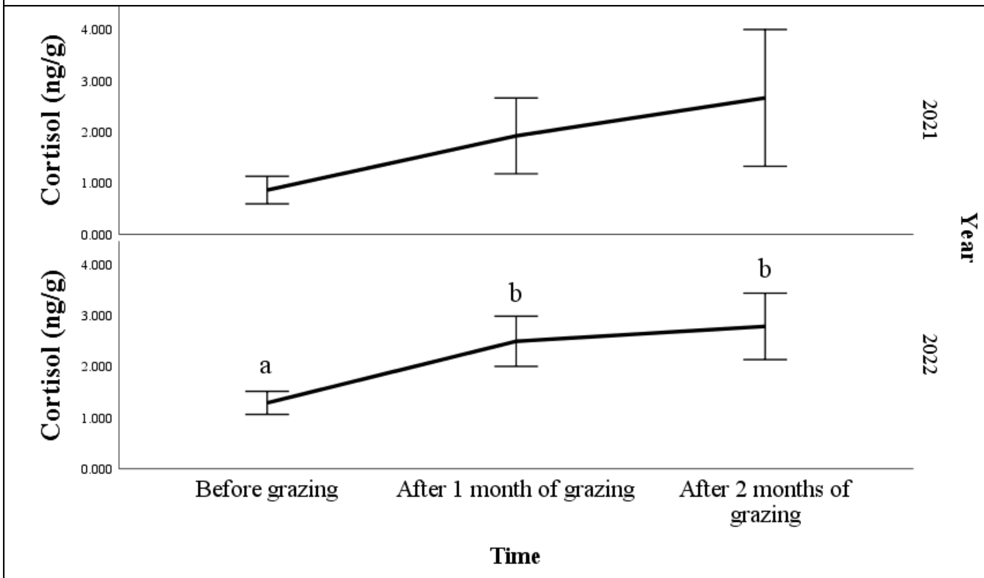


Supplementary Figure 2. Lombardy map showing the area of production of hay fed during winter season (red circle) from Pavia province in the Po River plain, and the area of alpine pasture grazed in summer season (blue circle) in Val Bregaglia (Sondrio province).

Descriptive statistics and multiple comparisons	**P value**		
	Time	**Year**	**Time x year**
	0.014	0.867	0.775
	0.602	0.664	0.798

	0.001	0.786	0.539
	0.163	0.669	0.865
	0.001	0.158	0.145

	0.002	0.700	0.068
	0.161	0.511	0.988
	0.001	0.449	0.561

	0.412	0.276	0.504
	0.524	0.133	0.430
	0.010	0.195	0.980

Supplementary Figure 3. Changes in hair concentrations of trace elements and heavy metals, and cortisol during transhumance stratified by year, and results of the factorial models and pairwise comparisons.

Supplementary Table 1. ICP-OES device parameters for determination of trace and toxic elements.

Parameteres	Assigned value
Plasma gas flow rate	15 L/min
Argon carrier flow rate	0.5 L/min
Sample flow rate	1.51 L/min
The speed of peristaltic pump	100 rpm
RF Power	1150 W

Supplementary Table 2. Wave lengths used in the analysis for each element.

Elements	Wave Length (nm)
Aluminum (Al)	167.070
Arsenic (As)	189.042
Boron (B)	208.959
Chromium (Cr)	267.716
Copper (Cu)	324.754
Iron (Fe)	259.940
Magnesium (Mg)	285.213
Nickel (Ni)	341.476
Lead (Pb)	220.353
Zinc (Zn)	206.200

Supplementary Table 3. Content of the elements and heavy metals assessed in the hair of Frisa goats (n=24) collected one day before vertical transhumance (before grazing), one month, and two months after the vertical transhumance. Values are means and standard errors (SE).

	Time					
	Before grazing		**After 1 month of grazing**		**After 2 months of grazing**	
Parameter	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
As (µg/g)	2.75	0.43	4.23	0.46	4.24	0.35
Cr (µg/g)	2.46	0.29	2.18	0.17	2.34	0.20
Cu (µg/g)	6.45	0.43	5.76	0.21	6.85	0.29
B (mg/g)	1.00	0.10	0.98	0.06	0.83	0.06
Mg (mg/g)	0.45	0.02	0.43	0.02	0.38	0.01
Zn (mg/g)	0.09	0.00	0.09	0.00	0.08	0.00
Ni (µg/g)	1.38	0.23	0.93	0.24	1.32	0.28
Pb (µg/g)	1.01	0.19	0.75	0.13	0.94	0.18
Al (mg/g)	0.12	0.01	0.16	0.03	0.08	0.01
Fe (mg/g)	0.27	0.05	0.19	0.03	0.20	0.06

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4 GENERAL DISCUSSION AND CONCLUSIONS

The world's livestock patrimony represents a vital tool to face the humanity's future challenges: from climate change and demographic increase to the cyclical events impacting the flora and fauna on Earth, such as infectious disease epidemics. Indeed, the peculiar characteristics of each domesticated species and breed, resulting from the adaptation over the centuries to different environments and human-animal relations, can be exploited to ensure food security for the largest possible human population. Different characteristics can be used with this aim, starting, for example, with adaptation to drought environments with water and good-quality-fodder scarcity. Moreover, disease resistance/resilience will also be an extremely important trait in order to optimize the breeding systems and mitigate the phenomenon of antibiotic resistance. Thus, preserving local breeds, with their characteristic greater rusticity, frugality, and ability in the conversion of food of low nutritional value, is of crucial importance.

In this project, several physiological aspects were investigated in different local and transboundary goat breeds. In particular, the autochthonous breeds investigated were those mainly bred in Lombardy in the Central-Western Alps, characterized by notably low populations and often experiencing a declining trend. The cosmopolitan breed that was used in our studies to compare with local breeds was the Camosciata delle Alpi, a dairy breed that is among the most widespread throughout Italy and the world, which originates from Switzerland.

Regarding the productivity of goat breeds, our results showed a clear lower milk yield of the local breeds, which was already known. However, from a qualitative point of view, as has already been demonstrated in other local breeds from Central and Southern Italy, the autochthonous breeds exhibit unique characteristics that impact the final product. The higher lactose content found in the milk of the Verzasca goat in our study, compared to the cosmopolitan Camosciata, is an interesting result considering that the same observation had previously been made when comparing the cosmopolitan Saanen breed with local breeds such as the Garganica, Girgentana or the Rossa Mediterranea (Currò et al. 2019). It is hypothesized that, in general, the quality differences can be ascribed mainly to artificial selection processes aimed at increasing yields in transboundary breeds, which result in a reduction of their milk components. However, further investigations should be carried out to identify the physiological mechanisms that led to parallel differences between the Italian local breeds evaluated until today.

From a reproductive point of view, our study showed that the probable common origin of the breeds we investigated, belonging to the cluster of European-Alpine breeds, determined a close genetic similarity of the genes involved in the reproductive seasonality regulation. However, many aspects remain to be investigated, starting with the disparity in the number of average kids born alive per goat, as evident from the data we collected and showed in the study for the different breeds. Although the genetic component influence is known, the type of farming system could also be important, which in this case could lead to a penalization of local breeds that, having lower energy-content ratio available, are not able to sustain the growth of the same number of fetuses as in semi-intensive or intensive farmed transboundary breeds.

Finally, with regard to metabolic and immunological aspects, we have conducted several investigations in this interesting field, and further studies are in progress. Regarding the comparison of both the basic composition and the concentration of some of the most important immunological molecules in the colostrum of local and cosmopolitan breeds, we have shown an interesting variability in the composition of this product between breeds, which could be mainly related to differences in the breeding system and the genetic component, related to the natural selection processes and the aptitude (dairy or meat) of the animals. In general, local breeds showed a higher quality of colostrum, which confers greater hardiness and rusticity to their kids. Similarly, positive results were obtained in the investigation of haematological parameters conducted on Verzasca and Camosciata breeds kept under the same farming system. This study highlighted how the differences in haematological parameters are closely linked to breed genetics, in turn resulting from the different artificial and natural selection to which they have been subjected. Furthermore, some of the observed differences were closely linked to a potential resistance/resilience of the local breed to the most common gastrointestinal endoparasites of small ruminants, such as *H. contortus* (Zanzani et al. 2020). Furthermore, this increased adaptation to environmental stressors was also confirmed by the N/L ratio. In this regard, the study conducted on Frisa goats investigated the adaptation to environmental stressors, by assessing the activation of the hypothalamic-pituitary-adrenal axis due to the change in farming environment during vertical transhumance of the flock. The results showed that goats are more stimulated at the axis level when reared extensively on alpine pastures, although these stimuli are not necessarily intended as negative, and are probably mainly attributable to the greater need for movement in search of food, carried out voluntarily by the animals. In the same study, we also highlighted the important role, which could be exploited in the future in the One Heatlh perspective, of local breeds as bio-indicators of environmental contamination, specifically for heavy metals and trace elements. The close link between environment and local breeds is what makes these animals so valuable for environmental biomonitoring.

In conclusion, this project has demonstrated, with a multidisciplinary approach, the great variability of physiological adaptations existing between different breeds, and the greater rusticity and hardness exhibited by local breeds compared to transboundary breeds. Numerous aspects remain to be investigated, considering both the physiological aspects and the breeds involved. The populations of local breeds are increasingly shrinking in numbers, and for this reason it is necessary to outline as quickly as possible a solid and concrete plan aimed at their protection and investigation. Certainly, one of the first steps that must be taken is the characterisation of the products (meat, milk, and cheese), in order to economically sustain the breeders, and the specific adaptations to the environment of each local breed. Our research group is currently already engaged in studying the adaptation during the transition period by comparing the blood metabolic profile of different local and cosmopolitan breeds. In addition, a characterisation of udder pathogens is also in progress. Although our results are very preliminary, we hope for more extensive research in this field in the future.

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