UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

BLOOD HEMATOLOGY AND BIOCHEMISTRY IN IBERIAN CATTLE BREEDS

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Abstract

The evaluation of certain biochemical variables such as blood concentrations are useful not only for the early detection of diseases, but also to study animal metabolism and related with animal performance. Information on blood metabolite concentrations in autochthonous and endangered breeds are thus of particular relevance. In some Iberian breeds the basic values of these concentrations are not defined. Therefore, the main objective of this dissertation was to establish standard values for plasma metabolites in Iberian cattle breeds farmed in extensive systems. For this, selected hematological and biochemical parameters of a total of 311 animals of different breeds (Avileña, Lidia and Morucha), ages and sexes were analyzed. Regarding the results, we could verify that the Morucha breed had the highest red blood cells count and hematocrit percentage while Lidia had the highest values of glucose and ALP. In sex terms, males presented higher values of white blood cells and total proteins and females the highest values of albumin and glucose. In the matter of age, older animals had significantly higher creatinine concentrations.

Keywords: metabolism; blood parameters; autochthonous; hematology; biochemistry

Resumo

O estudo de certas variáveis bioquímicas no sangue é importante não só para a deteção precoce de doenças, mas também para estudar o metabolismo e relacioná-lo com a performance dos animais. Esse estudo assume ainda maior importância quando falamos de raças autóctones, muitas em perigo de extinção. Em algumas raças ibéricas os valores base dessas concentrações não se encontram bem definidos. Perante isto, o principal objetivo desta dissertação visou estabelecer valores padrão para os metabolitos plasmáticos em raças bovinas do tronco ibérico exploradas em sistemas extensivos. Para isso, foram analisados alguns parâmetros hematológicos e bioquímicos de um total de 311 animais de diferentes raças (Avileña, Lidia e Morucha), idades e sexos. Pudemos verificar que a raça Morucha obteve o maior número de glóbulos vermelhos bem como a maior percentagem de hematócrito, enquanto a raça de Lide teve os maiores valores de glucose e ALP. Em termos de sexo, os machos apresentaram valores mais elevados de glóbulos brancos e proteínas totais e as fêmeas valores superiores de albumina e glucose. Em relação à idade, os animais mais velhos apresentaram concentrações significativamente superiores de creatinina.

Palavras-chave: metabolismo; parâmetros sanguíneos; autóctones; hematologia; bioquímica

Resumen

El estudio de ciertos parámetros bioquímicos como las concentraciones sanguíneas de algunos metabolitos son útiles, no solo para la detección temprana de enfermedades, sino también para estudiar el metabolismo animal y sus producciones. Este estudio tiene mayor importancia cuando hablamos de razas autóctonas en peligro de extinción. En algunas razas ibéricas los valores de referencia de estas concentraciones no están definidos, por lo que su interpretación resulta difícil. Ante esto, el principal objetivo de esta Memoria fue establecer valores estándar para los diferentes valores sanguíneos en razas bovinas del tronco ibérico explotadas en sistemas extensivos. Por ello, se analizaron algunos parámetros hematológicos y bioquímicos de un total de 311 animales de diferentes razas (Avileña, Lidia y Morucha), con diferentes edades y de ambos sexos. Pudimos verificar que la raza Morucha tenía el conteo de glóbulos rojos y el porcentaje de hematocrito más alto, mientras que la de Lidia tenía los valores más altos de glucosa y ALP. En términos de sexo, los machos presentaron valores más altos de glóbulos blancos y proteínas totales y las hembras los valores más altos de albúmina y glucosa. En cuanto a la edad, los animales más viejos presentaron concentraciones de creatinina significativamente más altas.

Palabras clave: metabolismo; parámetros sanguíneos; autóctonas; hematología; bioquímica

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Abbreviations and Acronyms list

Alb - albumins

ALP – alkaline phosphatase

ALT – alanine aminotransferase

AST (SGOT) - aspartate aminotransferase

BHBA - β-hydroxybutyrate

BUN- blood urea nitrogen

Ca - calcium

Chol - cholesterol

CK – creatine kinase

Cr - chromium

Cre - creatinine

Cu - copper

DGAV - *Direção Geral De Alimentação e Veterinária*

Fe - iron

FFA - free fatty acids

FSH - follicle stimulating hormone

GGT - gamma-glutamyl transferase

Glu - glucose

Hb - hemoglobin

Ht or PCV - hematocrit

ICP- AES - plasma atomic emission spectrometry

ICP- MS - inductively coupled plasma mass spectrometry

IGF-1 - Insulin-like growth factor 1

K - potassium

L.T.I – *Laboratório de Técnicas Instrumentales*

LDH - lactic dehydrogenase

MAPA - *Ministerio de Agricultura, Pesca e Alimentación*

MCH - mean corpuscular hemoglobin

MCHC - mean corpuscular hemoglobin concentration

MCV - medium corpuscular volume

Mg - magnesium

Na - sodium

NEFA – non-esterified fatty acids

Ni - nickel

NW - northwest

P - phosphorus

Plq - platelets

RBC – red blood cells

Se - selenium

TP – total proteins

WBC - white blood cells

Zn - zinc

1.Introduction

The availability of information on metabolic indicators is crucial to monitor the physiological, health and production status in all animal species. Furthermore, they allow to promptly intervene in the case of metabolic diseases, nutritional imbalances as well as different aspects in the context of animal production. A common method to monitor the metabolic health and nutritional status of the animals is metabolic profiling, that is based on the identification and quantification of a set of blood metabolites. These include a vast array of different metabolites such as glucose or calcium, among many others (Benedet et al., 2020).

Although in the bovine species, particularly in dairy cattle, metabolic profiling is frequently used and numerous expectable concentration ranges have been defined for a vast array of metabolites in the blood, such information is generally scarce for many of the different autochthonous breeds that are frequently understudied and underrepresented. This study aims thus to fill this knowledge gap, particularly what concerns the autochthonous or indigenous breeds, more specifically the Iberian breeds that are present in both Portugal and Spain. These breeds have decreasing inventories since the mechanization of agriculture. They have nonetheless remained unchanged for centuries, being among the oldest and most resilient cattle breeds in the world. They are very different in temperament, productive performance and even certain metabolic and physiological aspects, from the majority of “mainstream” cattle breeds like the Holstein-Friesian, the Angus or the Charolais that have been thoroughly characterized regarding blood metabolites profiling. As such, in many of these Iberian breeds the expectable values of certain blood parameters are not at all established, which difficult both the management and the study of these animals (Carvalho, 2000).

The aim of this dissertation is to contribute to the establishment of the standard values for plasma metabolites in cattle breeds of Iberian lineage, that are produced in characteristic extensive systems like the *Montado*/*Dehesa*, so characteristic of some regions of the Iberian Peninsula.

2.Literature review

2.1.Cattle´s origin and Domestication

Domestic cattle belong to the *Bovidae* family, and the *Artyodactila* order that includes other genus such as the bison and several buffalo species. The first bovid emerged on the Miocene, approximately 20 million years ago (Iglesias,1989). From that point, several species have developed, some of which became extinct.

Among such species, aurochs (*Bos primigenius*) stand out. The huge wild cattle (Figure 1) that existed during the entire pre-history were the wild ancestral of the domestic cattle (Epstein and Mason, 1984). The aurochs had their origin in Asia and expanded across Europe and North Africa, with fossil records of these animals from the Iberian Peninsula to China (Epstein and Mason, 1984). This formerly widespread wild species became extinct in the 17th Century, with the last recorded herd found in 1627 in Poland (Götherström et al., 2005).

Figure 1- Auroch artisitic depiction
Source: Wikipedia, 2021

There are currently two recognized forms of domestic cattle: the taurine cattle (*Bos taurus*) - without hump, from Europe, Africa (West) and North Asia; and the zebu cattle (*Bos indicus*) - with a cervo-thoraxic hump and originating from the South of Asia and Africa (Carvalho, 2000). *Bos taurus* animals can be divided into two groups: on one hand the *Bos taurus* animals from continental Europe - heavy animals with leaner carcasses and less intramuscular fat; On the other hand, the *Bos taurus* animals from the British Isles - smaller animals with fattier carcasses (less muscled carcasses) and more intramuscular fat (Almeida, 2018)

Domestication of animals, such as cattle, has been very important in the evolution of our civilization, contributing decisively in some essential aspects for the subsistence of Human beings. Firstly, domesticated animals are a stable source of food (meat and milk) and other animal

products such as leather, hides and even dung. On the other hand, before the mechanization of agriculture and the industrial revolution, a large part of the animals, such as cattle, donkeys and horses were used for agricultural work, performing a variety of activities such as transport, plowing and riding (Zhang et al., 2020).

The beginning of cattle domestication took place about 8 000 to 10 000 years ago, in the Neolithic Fertile Crescent (Epstein, 1971). In this first phase, somewhere in Southwest Asia, domestication was done from wild populations of aurochs, giving rise to the *Bos taurus* cattle. After its domestication, the expansion of *Bos taurus* was made very quickly, mainly according to two migratory routes: on one hand from Palestine through Egypt to Northwest Africa, on the other hand from Minor Asia through the Balkans to the Iberian Peninsula (Epstein and Mason, 1984).

The second phase of the domestication process divides authors. For some of them, the origin of the zebu cattle is related with the expansion of *Bos taurus* to the east. According to these authors, this origin resulted from a selection process that occurred due to the need to adapt to hostile conditions and high temperatures existing in these areas (Epstein and Mason, 1984). In contrast, authors like MacHugh et al. (1997) support a different explanation that suggest that zebu cattle, *Bos indicus,* originated from a different ancestor.

Loftus et al. (1994) demonstrated through the analysis of the variation of the mitochondrial DNA sequence, the presence of two distinct lineages, one that included the taurine cattle and the other that included the Zebu cattle. This study suggests that the two groups diverged more than 200 000 years ago, providing strong evidence for the occurrence of two independent domestications. Besides these divergent domestications, it is also believed that these groups do not share a common ancestor. On one side, *Bos primigenius nomadicus* is assumed to be the zebu cattle ancestor, while the taurine cattle were generated from the *B. p. primigenius* (Loftus et al., 1994; Carvalho, 2000).

Although many authors defend that cattle domestication occurred in two major phases, a third domestication event has been hypothesized to have occurred somewhere in northeast Africa about 8 000 to 9 000 years ago. From that event resulted the divergent African taurine cattle (Pitt et al., 2019). This theory is essentially based in archeozoological and genetic evidence. In archeozoological terms, the evidence is based on data resulting from the comparison between osteological analyses of ancient wild and domestic cattle off Europe, Asia and Africa (Applegate et al., 2001). In genetic terms, Bradley et al. (1996), using maternal mitochondrial DNA (mtDNA), concluded that the frequency of mitochondrial haplogroup T1 in African cattle is higher than is common in other regions. This conclusion allowed the authors to estimate that the separation between African and European taurine ancestors occurred earlier than the first domestication event in the Fertile Crescent (more specifically 22 000 to 26 000 years ago). This means that there was a local domestication of Africa taurine cattle and posterior mixture of Near East and the Indus Valley cattle in Africa (Pitt et al., 2019). Therefore, it is believed that this third domestication process was made from the Africa aurochs subspecies, *B.p. opisthonomus* (Carvalho, 2000).

2.2.Iberian Cattle Breeds Origin

It is generally assumed that most cattle breeds from the Iberian Peninsula are originated from the Near East, following several migration routes (Troy et al., 2001). It seems that human migration was the main cause of the introduction of these animals in the Peninsula, during the Neolithic period (Epstein and Mason, 1984). These migrations occurred essentially by two routes, one from the Southeast Asia, crossing the Balkan Peninsula to the Iberian Peninsula, and another by crossing the Strait of Gibraltar. In this route, people from North Africa, brought descendants of *B. p. opisthonomus* (Villena et al.,1999). Additionally, several invasions from the East, like the Phoenicians in 1000 b.C, the Greeks in 700 b.C and the Romans in 300 b.C. (Carvalho, 2000), contributed significantly for the diversification of cattle breeds, once the Aquitanian (*Bos tauros aquitanus*) and the Iberian (*Bos tauros ibericus*) lineages were originated as a consequence of their expansion (Vale, 1949).

Furthermore, Iberian Peninsula invasions through the Strait of Gibraltar also influenced the formation of other Iberian cattle breeds, such as the Mauritania lineage (Vale, 1949), because of the Carthaginian and Moorish occupations in respectively 500 b.C and in 711 a.C (Epstein and Mason, 1984).

Over the centuries, the Iberian cattle populations occupied different geographical areas. This mechanism of natural selection imposed by the different environmental conditions combined with the alternation between migration and stability periods promoted a selection of different animal characteristics, either morphological or productive (Carvalho, 2000). Until the 19th century, the cattle were essentially used for labor, especially in agriculture. With the industrial revolution and the mechanization of the agriculture, and the growing need to supply large cities, the use of cattle for working decreased and animal selection turned to be based on the productivity of the animals (meat and milk) and not only on its capacity to work (Epstein and Mason, 1984; Carvalho, 2000). This selection method led to a sharp reduction of many breeds. Furthermore, the introduction of new reproductive techniques, like artificial insemination, and the increment of crossbreeding, intensified this decrease, leading to many autochthonous breeds extinction (Moazami-Goudarzi et al., 1997).

Nowadays, according to the Portuguese DGAV (*Direção Geral De Alimentação e Veterinária*, 2021) and the Spanish MAPA (*Ministerio de Agricultura, Pesca e Alimentación*, 2021) there are about 55 Iberian native breeds, 15 Portuguese and 40 Spanish. Among the Spanish breeds, 32 of the 40 breeds are endangered.

It is important to note that some of these breeds, although they are considered autochthonous, are actually foreign breeds, brought from other countries and that have been perfectly acclimated to the environment where they are raised.

2.3.The Iberian Cattle Breeds

Among the 55 autochthonous cattle breeds existing in the Iberian Peninsula, we made a selection based on criteria related to the morphological characteristics as well as the production systems of these animals. Thus, in the framework of this introduction, the chosen breeds were those that most resemble the fighting bull breed (In Spanish the Lidia and in Portuguese the Brava or Lide). These ancient rustic breeds are produced in extensive production systems in less-favored areas, with the ability to take advantage of less fertile land and withstand harsher climates. Some were formerly used as draught animals. Presently they are used as maternal lines in crossbreeding with industrial breeds, although they do not always improve meat or dairy production.

Using this criteria, 19 breeds were selected, 15 Spanish: Alistana-Sanabresa, Avileña Negra Ibérica, Avileña-Negra Ibérica (Bociblanca), Berrenda en Colorado, Berrenda en Negro, Blanca Cacereña, Cardena Andaluza, Lídia, Marismeña, Morucha, Morucha Negra, Negra Andaluza, Pajuna, Sayaguesa, Serrana Negra; and 4 Portuguese: Lide, Lide dos Açores, Mertolenga and Preta.

2.3.1.Spanish breeds

In figure 2 it is represented the provinces where each Spanish breed is distributed in greater number. After the map we made a brief description of each breed.

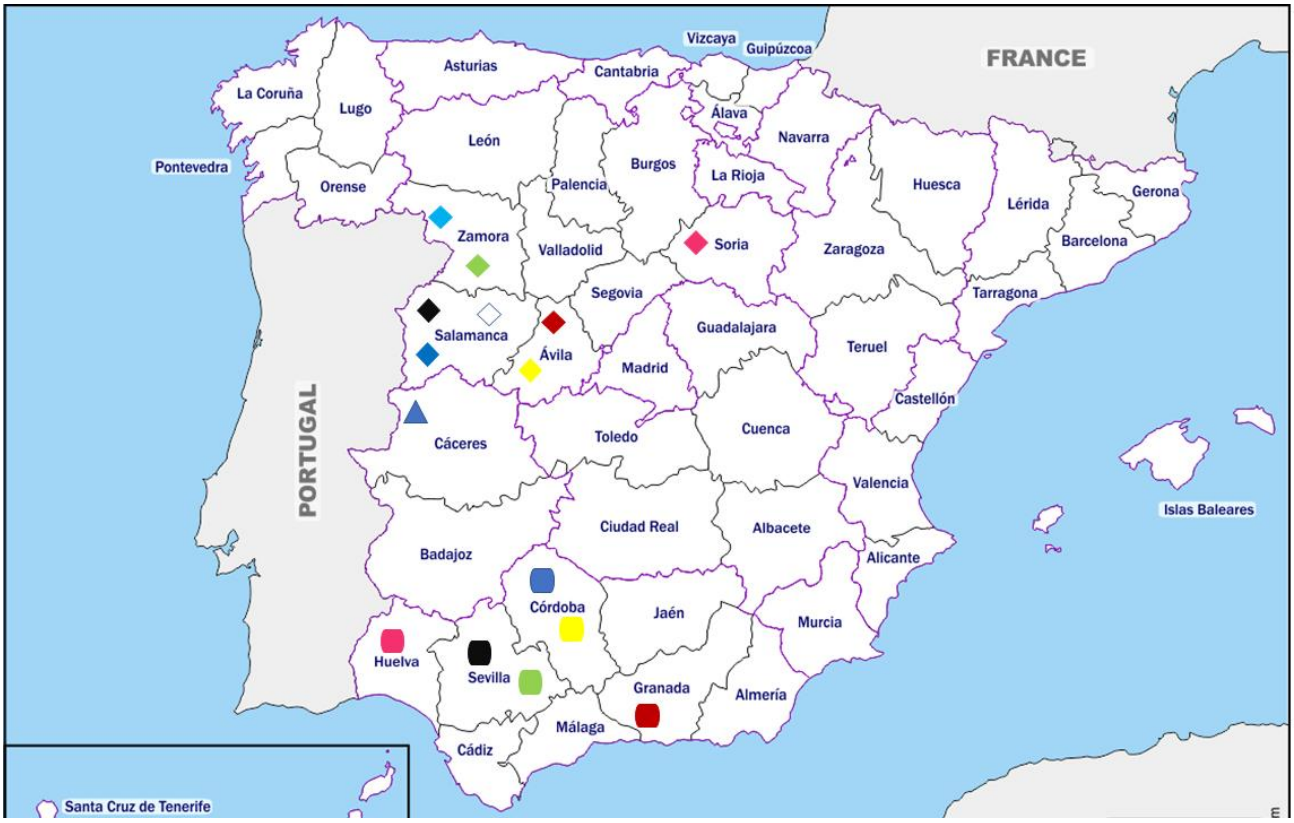

Figure 2 – Distribution of the Spanish chosen breeds considering the provinces where each breed is distributed in greater number..

- Alistana-Sanabresa; - Sayaguesa; - Serrana Negra; - Morucha; - Berrenda en Colorado; - Morucha Negra; , -Avileña-Negra Ibérica; - Avileña-Negra Ibérica Bociblanca; - Blanca Cacereña; - Marismeña; - Berrenda en Negro

- Lidia; - Negra Andaluza; - Cárdena Andaluza; - Pajuna

Map source: https://d-maps.com/carte.php?num_car=5674&lang=pt , 2021

Alistana-Sanabresa

The Alistana-Sanabresa (Figure 3) has its origin in the Cantabrian lineage (*Bos taurus cantabricus*) which populated the lands of the northwest of Castilla & Léon and east of Galicia. This cattle breed belongs to the group of animals that were initially called "*Morenas do Noroeste"* which included almost all brown breeds existing in Castilla & Léon like Sayaguesa Galician breeds as Limiana, Cachena, Caldelana, Frieiresa or Vianesa. In 2020 this breed consisted of about

2693 animals distributed almost exclusively in Zamora. Alistana-Sanabresa is a very rustic breed, able to adapt to poor soils and extreme climate conditions. In feeding terms, they consume a wide variety of pastures, fruits and leaves. This allows their extensive production throughout the year without the need for supplementation, except of course in situations of extreme shortage. They are docile and easy to manage, which allows their production without many facilities need. Initially they were destinated to agricultural work, while the meat and milk production was minimal. One of the main characteristics of this breed is its well-marked sexual dimorphism as well as its concave profile. They are brown animals with some variations according to sex or age (Garcia, 2009a).

Figure 3 – Alistana Sanabresa cow and calf
Source: MAPA, 2021

Avileña-Negra Ibérica

The origin of Avileña-negra ibérica (Figure 4) is associated with the Iberian cattle that inhabited the mountain areas of the peninsular center (*Bos taurus ibericus*). From the second half of the 19th century the geographical area occupied by the black cattle was restricted to the Central *Meseta* plateau, giving rise to the so-called Serrana group, made up of animals with their names depending on the geographical area in which they were located. It is estimated that there are about 155 000 animals, essentially distributed in mountainous areas that are difficult for other breeds to use (Serra Morena, Serra Cameros, etc.). It is also associated with transhumance systems in the provinces of Central and Southeast Spain: Castilla-La Mancha, Extremadura, Castilla y León, Madrid and to a lower extent in Andalusia. They are very rustic animals, with high longevity and an ability to cover large distances in search for food. They are also animals with great capacity to use poor pastures, mainly in mountain areas, and even browse (they eat leaves and tender shoots of trees), having at the same time a meat production with high performance and high quality. The ability to ingest branches and shrubs is especially important in fire fighting. In morphological terms this breed is composed by black animals characterized by having a strong bone structure and joints which allows them to travel great distances in mountainous environments (Garcia, 2009b).

Figure 4- Avileña-Negra Ibérica bull
Source: MAPA, 2021

Avileña-Negra Ibérica (Bociblanca)

The Avileña-Negra Iberica (bociblanca) (Figure 5) is very similar to the Avileña-Negra Ibérica breed and can be considered as a variant of this one. Both breeds have the same origin as well as the same distribution among the country. In regard of the number of animals, in 2019 there were about 1013. The ecology and the management of both breeds are very similar. The only characteristic that set them apart is the color of its coat. This difference concerns the presence of white strip around the mouth and nostrils (Herraiz-Espinoza, 2009).

Figure 5 – Avileña-Negra Ibérica (Bociblanca) cow and calf
Source: MAPA, 2021

Berrenda en Colorado

According to Sánchez Belda, 2002, everything indicates that the Berrenda en Colorado's (Figure 6) ascendant is the *Bos tauros desertorum* that predominated in southwestern region of the Iberian Peninsula. In 2019 there were 6179 Berrenda en Colorado that were distributed throughout many regions in Spain, mainly the Andalusia and Castilla & Léon regions. The

Berrenda en Colorado is a rustic breed, well adapted to extensive and super-extensive systems as well as to adverse climates and food shortage. It has a characteristic color, white with brown stains, which usually cover the head and the four paws of the animals. This breed is extremely similar to the Mertolenga cattle breed of Southern Portugal (Jurado et al., 2009a).

These cattle are used in two different ways: on the one hand, the females are used in meat production due to their excellent maternal characteristics; on the other hand, since they are docile and easy-to-manage, males are used as auxiliaries in the management of lidia cattle, working as *cabrestos*.

Figure 6- Berrenda en Colorado herd
Source: MAPA, 2021

Berrenda en Negro

The Berrenda en Negro breed (Figure 7) has its origin in the Iberian lineage. In relation to its phylogeny, it was concluded that it has many similarities with the Negra Andaluza breed. Its main distribution zone is the mountainous areas of Andalusia and in 2019 there were about 3455 animals. Both in ecological and management levels this breed is very similar to the Berrenda en Colorado. Both breeds are used in meat production as well as in the management of lidia cattle. In morphological terms there are also large similarities between the 2 breeds except in coat's color. Whlie Berrenda en Colorado has a white color with brown spots, berrenda en negro, as the name implies, has a white color with black spots (Jurado et al., 2009b).

Figure 7 – Berrenda en Negro bull
Source: MAPA,2021

Blanca Cacereña

The origin of this breed remains unknown, although authors such as Sanchez Belda (1981), consider it as the oldest cattle breed in Spain. There are two major theories about the introduction of the Blanca Cacereña (Figure 8) in the Iberian Peninsula. On the one hand, it is possible that African peoples, based on cave paintings of Tassi Li (Egypt), made its introduction. On the other hand, it is supposed that it might have been the Romans who brought this breed into the Iberian Peninsula, based on the documents that mention the sacrifices of white calves during the festivals in honor of the god Jupiter (Bergua, 1977 cited by Bartolomé-Garcia et al., 2009). In 2019 there were about 892 animals distributed exclusively in Extremadura, more specifically in small herds in the provinces of Cáceres and Badajoz. The Blanca Cacereña breed has a primitive morphology - rustic and strong – due to their poor development. They are adapted to extensive grazing systems of the Extremadura region. Their meat production falls far short of other breeds even when compared to other native breeds, making the Blanca Cacereña a less and less used breed (Bartolomé-Garcia et al., 2009).

Figure 8- Blanca Cacereña cow and calves
Source: MAPA, 2021

Cardena Andaluza

The Cardena Andaluza (Figure 9) is characterized by excellent maternal characteristics despite being a slow growing breed. Its origin derives from the Iberian lineage (*Bos tauros ibericus*) and is currently distributed throughout the region of Andalusia, more specifically in the provinces of Cordoba and Huelva. In 2019 only 862 animals were registered, making Cardena Andaluza an endangered breed. It is a rustic breed, well adapted to dry and warm climates (such as the areas of Andalusia mountains) with fibrous pastures and water scarcity, which often forces these animals to move for a large part of the day, under very high temperatures in search of water. Its name is associated the coat color (grey shades, *cardena* in Spanish) and its distribution area (Jurado et al., 2009c).

Figure 9 – Cardena Andaluza female
Source: MAPA,2021

Lidia or Brava

It really is not a single breed, but a racial grouping. The origin of the Lidia breed (Figure 10) is difficult to ascertain given the large difference between the animals of the same breed. It is believed that it arose from crosses of several primitive native animals (from all the lineages that contributed to the formation of the Iberian breeds) and was exclusively selected for its aggressiveness (*bravura*) to taurine festivals and not for its capacity to produce meat (Garcia, 2009c). The Lidia breed is the second most important bovine breed in the Spanish census. In 2019 there were 219 053 Lidia animals, mainly located in the Andaluzia, Castilla & Léon, Extremadura and Castilla-la-Mancha regions.

The Lidia breed is perhaps the cattle breed better suited to the Spanish interior extensive systems. These animals have great rusticity and live in wild environments, needing large areas to exercise and develop the breed's typical behaviors. The Lidia livestock areas tend to be isolated from the population to reduce human contact as much as possible, in order to prevent the development of abnormal behavior in animals. For this reason these animals are reared on large grasslands located in mountains and hills. The feeding is highly dependent on the rainfall, mainly based on the use of natural resources from pastures. Their feed is supplemented in scarcity periods, in weaning, in stallions bulls, as well as in the last year before the fight, which is called finish (*acabado*). This supplementation is made from forages or concentrates that can be produced on the farm itself or purchased (Garcia, 2009c).

In morphological terms, there is a very marked sexual dimorphism and a huge color variation (where black is the most common), that is why we speak of *encastes* or *castas,* which we could associate to breeds within the racial group. They are small well-proportioned animals and do not have a very prominent skeleton. Males reach an average of 500 kg while females reach 300 kg. A greater harmony in the male proportionality and body and muscle development compared to females could be explained by the fact that the pressure of morphological genetic selection has been exerted on the bulls due to their greater economic value (Lomillos and Alonso,

2020). In these animals, there are internal morphological differences between the different genetic lines of the breed, forming several different *castas*, which can be considered as subraces or perhaps even strains within the Lidia breed. Currently, according to the *Centro Etnógrafrico y Bibliográfico virtual del toro de Lidia* 2021, there are 5 different *castas: Cabrera, Gallardo, Navarra, Vazqueña* and *Vistahermoza.*

Figure 10 - Lidia Bull
Source: Instagram of @ganaderiajandilla

Marismeña

The Marismeñas's phylogenetic ancestry is unknown, although it is supposed to be linked to the first forms that derived from the wild Uro. From these, *Bos tauros tartesus* was formed, which according to Sanchez Belda, 2002, is the direct ancestor of this breed. In 2019 there were about 3650 animals, all located in Doñana natural park, in Andalusia. In this park, animals remain in a wild state, without any control in crossbreeding with other breeds existents in the park. The Marismeña (Figure 11) animals are very identical to primitive breeds once they don´t have a great muscle mass or a strong bone structure. They also have a very thick skin and uniform red color, with the possibility of white stains on the lower body areas (Jurado et al., 2009d).

Figure 11 – Marismeña bull
Source: MAPA,2021

Morucha

The Morucha breed (Figure 12) has its origin in Iberian lineage (*Bos taurus ibericus*). In ancient times, it was appreciated by its triple capacity for work-*lidia*-meat, until the demand of the market forced its specialization. Despite this specialization, a part of the inventory was crossed with *lidia* animals in order to take advantage of the characteristics of these animals. In 2019 this breed had a total of 16 165 animals, mainly distributed throughout Castilla & Léon, especially in Salamanca province, where more than 50% of the morucha breed inhabits. Despite these numbers, there are records of these animals in other regions such as Extremadura, Castilla la Mancha and Madrid.

The Morucha is a very rustic breed, able to take advantages of the pastures among the Central Meseta. Furthermore, is characterized by its great maternal instinct, which combined with the calving ease and the high fertility make these animals an excellent maternal line for meat production. As regards its color, it can oscillate between shades of grey since there is a Morucha breed variety - the Morucha Negra breed - in which the animals are black. Males reach weights of 900 kg while females record weights around 500 kg. These animals are generally slaughtered at 12/13 months, with approximately 225 kg and 51% of carcass yield. Since 1993, work has been carried out on the genetics improvement scheme of the Morucha breed, with the objective to achieve improved performances and at the same time trying to preserve its rusticity and its management system

The Morucha's are produced in large meadows, being perfectly well adapted to this scarce environment, rich in open spaces where animals select and assimilate nutrients throughout the long grazing periods. It is a difficult breed to handle, therefore a very interesting adaptation of these animals to marginal environments is the fact that they have the ability to defend themselves from wolf predation. In food terms, and like the Avileña breed, during the winter, they take advantage of the acorn and the brushwood, while in the spring they utilize the pastures and the wastelands (Garcia, 2009d).

Figure 12- Morucha breed animals
Source: MAPA, 2021

Morucha Negra

The Morucha Negra (Figure 13) is considered a variety of the Morucha breed. What set them apart is the coat's color, in this case, and as the name suggests, they are black. Counting 5069 in the year of 2019, mainly distributed along the Castilla & Léon region, and in smaller proportions in Extremadura and Madrid Region (Recio, 2009).

Figure 13 – Morucha Negra animals
Source: MAPA, 2021

Negra Andaluza

The Negra Andaluza breed (Figure 14) has its ascendancy in the Iberian lineage[6] (*Bos tauros ibericus*). Its name results from the combination of its coloration, black (*negra* in Spanish) and the area of the country where it is distributed, Andalusia. The animals of the Negra Andaluza breed have a straight profile, with low muscle mass and a strong bone structure, they have a docile character and temperament which allows an easy management by the producers (Jurado et al., 2009e). When it arrived at the Andalusia fields, this breed was seen as one of the most competent in terms of agricultural labor, standing out from other breeds due to its strength, rusticity and dynamism. Just as the vast majority of the native breeds, these animals are characterized by their rusticity, being able to take advantage of the scarce resources available (Jurado et al., 2009e). It is currently used in extensive systems, especially in industrial crosses with specialized breeds in meet production. In 2019 there were about 2096 animals of this breed, being distributed throughout the region of Andalusia, especially in Cordoba.

Figure 14 – Negra Andaluza animal
Source: MAPA, 2021

Pajuna

The Pajuna breed (Figure 15) is a poorly selected and very old breed whose origin remains uncertain. There are several theories about their ancestry, with authors claiming that this breed is descended from the Atlas African lineage and others attributing their ancestry to the Iberian lineage (*Bos tauros ibericus*) (Jurado et al., 2009f). The Pajuna breed is possibly the most resilient cattle breed in Spain. It is adapted to adverse conditions, taking advantage of the limited resources of Andalusia's mountains which can not be used by other breeds (Luque et al., 2006). In morphological terms it presents the characteristics of a typical mountain systems breed. They are usually animals with straight profile and medium proportions, with low muscle mass and a strong and massive bone structure. The Pajunas are dark brown animals with abrasions on the extremities (Jurado et al., 2009f). In 2019 there were 1223 Pajunas, distributed essentially among the Andalusia's mountains with a small group of animals in Castilla-La-Mancha.

Figure 15 – Pajuna cow
Source: MAPA,2021

Sayaguesa

Sánchez Belda, 1981, considers that the Sayaguesa breed (Figure 16) has its ascendancy in Iberian lineage, deriving from the *Bos taurus ibericus*, the black ancestral cattle from the central mountains of the Iberian Peninsula. In 2020, there were only 2385 animals, almost exclusively distributed in Castilla & Léon and a small group in Castilla-La-Mancha. They are animals produced mainly in small family plots that back in the days alternated the daytime grazing with the night stables. Nowadays they are produced according to an extensive system of permanent grazing and due to their rusticity; they are capable to support poor soils and severe climates. In morphological terms, they are docile animals with a strong bone structure and good muscle mass. The skin of the Sayaguesa breed is abundant and elastic which results in the appearance of jowls. Although its color is black, white areas may appear at the bottom of the animal's body and there may be some discoloration in the cow's udder (Garcia, 2009e).

Figure 16 – Sayaguesa cow
Source: MAPA, 2021

Serrana Negra

In general, the origin of the Serrana Negra breed (Figure 17) is attributed to the *Bos taurus ibericus* cattle, making this breed part of the Iberian lineage. Its domestication was made in the Roman era and since then this breed is adapted to mountainous terrains. The Serrana Negra is endangered and in 2020 there were only 564 animals, located in Castilla & León, in the Soria province' northwest. They are well adapted to mountainous areas with steep orography and high altitudes. These animals have a large longevity and resistance to more harsh climates. Due to their strong muscle structure and joints, they have the ability to move over large distances as well as a great capacity for traction. In morphological terms, the Serrana Negras are well proportioned, medium sized and straight profiled animals. Their coats color is uniform black, and they have a white border that surrounds the nose (*bociblanca*) (Garcia, 2009f).

Figure 17 – Serrana Negra cow

Source: MAPA,2021

2.3.2.Portuguese breeds

In figure 18 is represented the regions where each Portuguese breed is distributed in

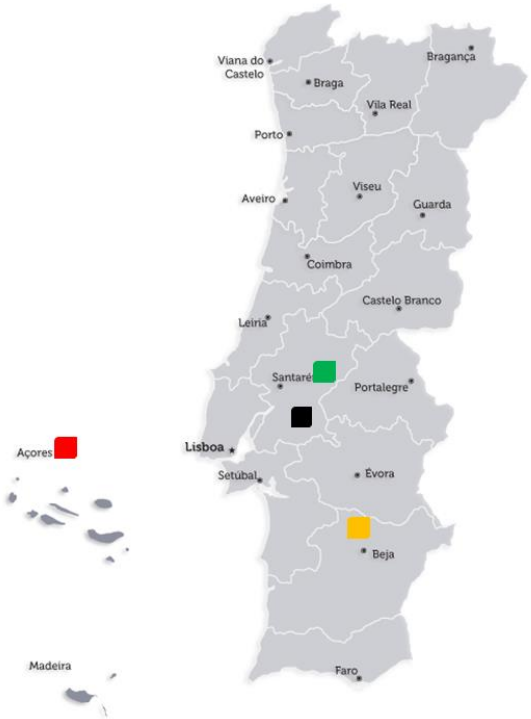

Figure 18 – Distribution of the Portuguese chosen breeds considering the regions where each breed predominates, according to SPREGA, 2021a.

- Brava de Lide dos Açores; - Brava de Lide; - Preta; - Mertolenga

Map source: https://cheveuxcrepusfrun.blogspot.com/2019/03/mapa-de-portugal-continental-e-ilhas.htm, 2021

Brava de Lide

Regarding its origin, it is known that the Brava de Lide (Figure 19), as well as all domestic cattle breeds, descended from the aurochs. These animals are used exclusively in tauromachic festivals, and, for that reason, their selection has been based on behavioral characteristics such as aggressiveness and not on productive ones related to meat and milk production (Gomes, 2016). Their production is done in extensive systems, with some infrastructures need for the adequate management of the animals. The Brava de Lide livestock areas tend to be isolated from the populations to reduce as much as possible the human contact in order to prevent the development of abnormal behaviors in these animals similarly to what was the described for the Spanish counterpart. Morphologically it is perhaps the smallest breed among the Portuguese native breeds. It is a rustic breed, made up of well-proportioned animals with a well-developed muscular skeleton (Gomes, 2016). In 2020 there were 10 456 animals distributed essentially among the Alentejo and Ribatejo regions (SPREGA, 2021b).

Figure 19 – Brava de Lide bull
Source : ruralbit, 2021

Brava de Lide dos Açores breed

There are some differences between the Brava de Lide and the Brava de Lide dos Azores (Figure 20) breeds. Firstly, its use. Although both are used in tauromachic festivals, the Azores breed is mainly used in the *tourada à corda*, a popular tauromachic tradition from the archipelago. In 2020 there were 1686 animals distributed throughout the Azores, especially in Terceira Island

(SPREGA, 2021c). In feeding terms, the favorable conditions that exist in the Azores such as the humid temperate climate and the high fertility soils allows the grazing during practically the whole year (Gomes, 2016).

Figure 20 – Brava de Lide dos Açores bull
Source: ruralbit, 2021

Mertolenga breed

There are several theories about the Mertolenga breed (Figure 21) ascendancy. When we explore the official website of the *Associação de Criadores de Bovinos Mertolengos,* we immediately come across three different views: some authors attribute their ascendancy to the *Bos taurus aquitanicus,* while others claim that these animals descend from the *Bos taurus turdetanus.* The third theory refers only to the *malhado* phenotype of Mertolengo, being its origin associated to the *Bos tauros ibericus* (Associação de Criadores de Bovinos Mertolengos, 2021). In 2020 there were 28151 Mertolenga cows distributed along the regions of Alentejo, Lisboa e Vale do Tejo, Beira Baixa and a small group in in São Miguel, Açores. The Mertolengas are produced in extensive systems and are used essentially as maternal line in crossbreeding with industrial breeds for meat production, or as *cabrestos*, aiding in the management of the Brava and Brava dos Açores breeds (similarly to the *berrenda en negro* and *berrenda en colorado*). Morphologically they are rustic animals, with a very pronounced sexual dimorphism. In phenotype terms, these animals have 3 groups: the *Vermelho* , the *Malhado* and the most common, the *Rosilho.*

Figure 21 – Mertolenga young bull
Source: ruralbit, 2021

Preta breed

The Preta breed (Figure 22) has its ascendancy in the *Bos taurus ibericus* (Iberian lineage). In the formation of this breed, there were crossings of a wide range of animals, with emphasis on the ones from the Ribatejo Lide breed as well as animals from Spanish breeds derived from the Black Iberian lineage. (IMAIAA, 1993, cited by Gonçalves and Rodrigues, 2002). In morphological terms they are black large animals with a strong bone structure. In 2020 there were 4039 animals distributed among the Alentejo, Lisboa e Vale do Tejo and Beira Baixa regions (SPREGA, 2021d). They can perfectly adapt to extensive meat production and due to the characteristics related to the high rusticity, they are preferential users of less-favored areas. Their diet is based on grazing, mainly of natural pastures, usually under the conditions of *montado*, cereal crops stubbles, acorns and tree trimming products. When the conditions require it, there is a need to administer supplements; usually it consists on straw and hay, and less commonly on concentrates. The Preta breed has excellent maternal characteristics, highlighting the ease of delivery and the good milk capacity. According to Gonçalves and Rodrigues, 2002, it is necessary to improve the inventory fertility rates for values that approximate those of other native breeds raised in the same system. This can be achieved by increasing the quality of the diet in food shortage times.

Figure 22 – Preta bull
Source: ruralbit, 2021

2.4.Blood metabolite profiling in cattle species

One of the most important fluids for the survival of cattle and most animals is blood. The amount of blood that each animal has in their body variates according to the specie, size and weight. Blood has three major functions that includes the transportation of gases, nutrients, hormones and the removal of waste products; the regulation of certain body mechanisms like the temperature and pH; and protection with the blood platelets that plays an important role in clotting process, essential to prevent blood loss (Menche, 2012).

The blood count and the analysis of some blood parameters (either hematological or biochemical) are very important to understand the animal metabolism and productivity. This analysis can be done through blood, serum (fluid obtained after blood centrifugation) or plasma (liquid part of blood).

Effect of Age

Age is one of the most important factors contributing to the occurrence of changes in the blood metabolic profile of animals. Over the years, several authors have studied these variations in cattle. In 1984, Barnes et al., measured multiple plasma metabolite concentrations in Holstein animals of different ages. The results showed that plasma prolactin levels increase as age progresses while glucagon concentrations tend to decrease. Insulin and glucose levels were greater in the 18 months heifers and lower in 24 months cows. Barnes et al also demonstrated that urea concentrations were higher in 24 months animals and lower in 12 months animals while the growth hormone concentrations reach its peak in younger animals (6 months), decreasing to 18 months and reaching again high values in 2-years animals. Finally, the values of free fatty acids were also evaluated, with an increase between 6 and 12 months of age, followed by a sharp decrease until the animals reach the 18 months. From that age, the fat free acid (FFA) values start to increase again.

Similarly, Peterson & Walden (1981) analyzing blood serum from 100 Holstein cows, concluded that glucose, cholesterol and lactate dehydrogenase (LDH) levels are strongly affected by age. According to these authors, while the glucose levels increase as age progresses, the cholesterol and LDH decrease. They could also check the influence of age on other blood metabolites, yet on a smaller scale (but in a significant way). Peterson & Walden (1981) concluded that in their study the total protein concentration was higher in older animals while the albumin, phosphorus, BUN (blood urea nitrogen), alkaline phosphatase and glutamic oxaloacetic transaminase concentrations decreased as age advances.

Mohri et al. (2007) worked with Holstein calves, in order to understand how some blood metabolites vary in the first 84 days of these animals' life. Among the many metabolites studied, they observed that age did not have a significant influence on the number of neutrophiles and monocytes, neither in the amounts of sodium, potassium, chloride and BUN. In parallel,

phosphorus and iron as well as albumin levels showed a significant increase from birth up to day 84. The calcium and glucose levels remained constant for the first two days and later a decrease was observed while the amount of magnesium decreased from day 14 and then slightly increased until the day 84. Changes in bilirubin concentrations were also evaluated, observing a decrease until day 14 and then remain low up to day 84. The last metabolites analyzed were the activity of alkaline phosphatase (ALP) and aspartate aminotransferase (AST). The activity of AST decreased from birth to day 14 and steadily increased up to day 84 while ALP activities decreased from birth to day 28 and then increased up to day 84.

Gregory et al. (2004) studied some biochemical blood parameters associated with the renal function. Using Jersey cows, they evaluated the influence of age on blood concentrations of creatine and urea. They were able to verify that serum urea levels increased gradual and significantly with age while serum creatine levels were lower in younger animals (less than 12 months) when compared to older animals. In 2001, Birgel et al. studied the leukogram in Jersey animals and evaluated how these values change with age. It was observed that the total number of leukocytes and lymphocytes increased until 12 months of age. Then, this number stabilized until 24 months, where it began to suffer a progressive decrease with advancing age.

The influence of age on the metabolic profile was also studied in the Shorthorn breed by Doornenbol et al. in 1988. In this study, it was concluded that age causes an increase in urea, total proteins and bilirubin concentrations as well as in serum activity of aspartate aminotransferase (AST) and lactate dehydrogenase (LDH). On the other hand, the animal's aging causes a decline in the calcium, phosphorus and alkaline phosphatase levels.

Studies on this field were also carried out on animals of Iberian breeds. Pereira et al. (1987) studied the metabolic changes associated with age development in the Spanish breed Blanca Cacereña. Regarding hematological values, they observed an increasing trend of MCV (Medium Corpuscular Volume), contrasting with the level of erythrocytes that shows an increase in their concentration with age. Hematocrits, hemoglobin and leukocytes levels show a similar variation, increasing until the age of 10 years, and from that point, their levels begin to decrease. In terms of the leukocyte formula, Pereira et al. concluded that as the animals age, the levels of lymphocytes, monocytes and eosinophils increase while the neutrophil content decreases.

Regarding the Iberian breeds, in 1982, Prieto et al. evaluated the blood parameters variations in Retinta cows of different ages. Regarding the hematological values, they observed that the highest hemoglobin values were found in younger animals (< 3 years) and, from there, a progressive decrease was observed according to age. They also observed that animals up to 5 years of age present very similar levels of erythrocytes and, from that age, these values begin to decrease. According to these authors, age did not influence leukocyte levels but caused a decrease in hematocrit concentrations although it was a minimal decrease. In relation to protein levels, Prieto et al. (1982) noted a slight increase in total protein contents while globulin concentrations decrease. Albumin values remain similar until the age of 4 years. From that point, there was an increase, and the higher value was found in animals over 7 years old. In this study,

changes in the glucose, total lipids and bilirubin contents were also evaluated. Glucose amounts increased progressively with advancing age, while for lipids there was a decrease of the values up to 5 years, followed by a gradual increase. Bilirubin levels decrease up to 5 years, then increase up to 7 and decrease again from that age. Finally, the behaviors of some blood ions were also investigated. Calcium and inorganic phosphorus concentrations decrease with age since higher values were found in younger animals. The magnesium content underwent an irregular fluctuation with advancing age, the highest value was found in animals between 4 and 5 years and the lowest value in animals older than 7 years.

Hernández (1992) was another author who studied the influence of age on the blood hematological levels of Iberian animals. Hernández evaluated these changes in the so-called Morenas del Noroeste that includes the Spanish breeds Cachena, Caldelana, Frieiresa, Limiana and Vianesa. Starting with the hematological values, more specifically the hemoglobin concentration, the author verified that it does not suffer significant changes with age, except in the Cachena breed, which after 10 years suffer a decrease of these levels. In relation to hematocrit levels they suffered a minimum decrease with age, although there was a residual increase after the animals reached 10 years. Hernández verified the existence of a negative correlation between erythrocyte and leukocyte levels and age, being the blood levels of these metabolites higher in younger animals. Despite this correlation, the author found that from the age of 10 years there was an increase in leukocyte concentrations (except in Cachena animals). In the matter of the results obtained related to the leukogram, he observed that in the Cachena, Frieiresa and Limiana breeds the neutrophil levels increased with age, contrasting with the Caldelana and Vianesa breeds where the correlation between these two variables was negative. In relation to eosinophil levels, in the Cachena, Caldelana and Limiana breeds these increased with age, unlike the remaining breeds where no significant differences were found. Hernández also observed a positive correlation between lymphocyte content and age in the Vianesa breed, contrasting with the other breeds studied where these concentrations decrease as the animals' age. Finally, as regards monocyte levels, in the Caldelana and Frieiresa breeds these levels increased with age, while in the other breeds the author verified the inverse, with a decrease in monocyte concentrations.

In addition to the metabolites already mentioned, Hernández analyzed the behavior of other serological parameters, some of which are presented in Table 1.

Table 1 - Serological variations of some metabolites with age

Metabolit	Cachena	Caldelana	Frieiresa	Limiana	Vianesa
Glucose	Decrease with age	Decrease with age	Decrease with age	Decrease with age	Decrease with age
Total Lipids	Decrease with age	Increase with age	Decrease with age	Decrease with age	Decrease with age
Triglyceride	Decrease with age	Decrease with age	Decrease with age	Decrease with age	Decrease with age
Cholesterol	Decrease with age	Increase with age	Increase with age	Decrease with age	Decrease with age
Free Fatty Acids	Irregular variations	Increase with age	Irregular variations	Irregular variations	Irregular variations
Creatinine	Decrease with age	Decrease with age	No changes observed	Increase with age	No changes observed
Urea	Irregular variations	Decrease with age	Irregular variations	Irregular variations	Irregular variations
Aspartate amino.	Increase with age	Increase with age	Slight decrease	Increase with age	Increase with age
Alanine amino.	Decrease with age	Decrease with age	Increase with age	Increase with age	Increase with age
Alkaline phosph.	Decrease with age	Decrease with age	Increase with age	Decrease with age	Decrease with age
Lactate dehydro.	Decrease with age	Increase with age	Increase with age	Increase with age	Increase with age
Glutamic latic des.	Decrease with age	Decrease with age	Increase with age	Decrease with age	Decrease with age

Source: Hernandez, 1992.

Regarding the total bilirubin levels, the author noticed a slight increase with age, until the animals reached 10 years, especially in the Vianesa breed. From the age of 10, the total bilirubin concentrations continue to increase in the Caldelana and Vianesa breeds, while they decrease in the Frieiresa and Cachena breeds and do not suffer variations in the Limiana. In the case of direct bilirubin this increased with age and, from the age of 10 years, the Caldelana breed was the only one that maintained a growth trend, unlike the others where Hernández verified a decrease in these contents. With regard to protein values, the author verified the existence of a positive correlation between total protein contents and age in the Cachena, Caldelana and Frieiresa breeds, contrasting with the Vianesa and Limiana, where he verified an inverse correlation. From the age of 10 irregular variations in total protein concentrations were observed, with the exception of the Limiana breed, where there was an increasing trend of these values. The changes in albumin concentrations were also studied and there was a positive correlation between these two variables in all breeds except in the Frieiresa breed where there is an inverse correlation. In older animals (more than 10 years) there is an increase of these values in the Cachena and Frieiresa breeds. In the remaining breeds there were irregular changes.

Finally, Hernández evaluated the oscillations caused by age in the blood concentrations of some minerals. Starting with calcium, he observed that in all breeds (except Frieiresa) sodium levels tend to decrease with age. The same happens with the values of inorganic phosphorus, where the author observed a higher concentration of this mineral in younger animals although, from the age of 10, there was a slight increase in the Frieiresa, Limiana and Vianesa breeds. In relation to the magnesium levels it was observed that there was a negative correlation with age in the Caldelana, Frieiresa, Vianesa and Limiana breeds, while in the Cachena breed the correlation between the magnesium levels and age was positive (in animals older than 10 years old, magnesium levels also start to decrease in the Cachena breed). The sodium concentrations suffered a decrease with age in the Cachena and Caldelana breeds contrasting with the other breeds where there was a minimal increase. Regarding potassium levels, these decreased as the age advances in the Caldelana, Frieiresa and Vianesa breeds, increased in the Limiana breed and showed minimal variations in Cachena. In the case of iron, Hernandez observed irregular

variations in all breeds, whereas in terms of copper, concentrations increased with age in the Frieiresa, Limiana and Vianesa breeds and decreased in the Caldelana and Cachena breeds, since in the last one there was an increase in concentrations after the animals reached the 10 years of age. Zinc concentrations increased in all breeds except Vianesa although after the animals reached 10 years of age these concentrations began to decrease slightly. Regarding the manganese levels the author observed that these increased with age in the Cachena and Vianesa breeds, increased until 10 years and then decreased in the Frieiresa and Limiana breeds and decreased until 10 years followed by an increase in the Caldelana breed. Hernandez concluded that age influences positively the molybdenum levels in the Caldelana, Limiana and Vianesa breeds. In the Cachena breed, observed that the concentrations of this mineral increase until 10 years and later suffer a decrease, while in the Frieiresa breed precisely observed the inverse. In relation to selenium, the author observed a negative correlation with age in the Frieiresa breed. In the Cachena, Limiana and Vianesa breeds selenium levels increased up to 10 years, suffering later a descent, observing the reverse in the Caldelana breed. Finally, Hernández concluded that age has no influence on cobalt concentration.

Effect of Sex

Walker et al. (2010) studied the influence of sex on some blood metabolites in Angus animals. The authors observed that serum IGF-1 as well as plasma glucose concentrations were greater in steers than in heifers. On the other hand, plasma urea concentrations were higher in females when compared to males. Finally, in this study, serum insulin levels were not affected by sex. With the same objective, Gregory et al. (2004) studied the alterations suffered by two metabolites associated with renal function - urea and creatine in Jersey animals. They observed that the serum urea levels in females were higher than those of males. In contrast, the sexual factor had no influence on serum creatine levels.

Doornenbal et al. (1988) also studied the influence of the sexual factor on blood concentrations of some metabolites in Shorthorn. In this study, the authors observed a higher concentration of hemoglobin, creatine, uric acid, and bilirubin, as well as a higher enzyme activity of alkaline phosphatase, aspartate aminotransferase and lactate hydrogenase in males when compared to females. In contrast, the reported blood levels of nitrogenous urea, glucose, cortisol, and hematocrits in females were higher than those found in males. In terms of protein content, the total protein levels recorded in males were slightly higher than in females, while albumin levels were found to be the opposite. Finally, the values of calcium and phosphorus were not affected by sex.

Doornenbal, in 1977 conducted another study related to blood parameters changes due to the influence of the sexual factor and where he also evaluated the differences between castrated and non-castrated males. Starting with the levels of hematocrits, the author found that these were higher in females compared to males, being the values of non-castrated animals

higher than the castrated ones). In relation to hemoglobin levels, these were lower in females compared to males, being the values of non-castrated animals higher than those of castrated animals. Regarding plasma sodium and potassium values, both were higher in females compared to males. Within males the values were higher in castrated animals. The corticosteroids concentrations were also evaluated, and the author found that the highest concentrations were observed in castrated males, followed by females, and the animals with the lowest levels were the non-castrated males.

In other studies, Birgel (1991) (cited by Hernández, 1992) studied the effect of the sexual factor in different blood parameters using Jersey animals in Brazil. In this work, the author observed that hemoglobin levels were higher in females compared to males, contrasting with the levels of hematocrits and erythrocytes that showed to be higher in males. Birgel also concluded that there were no significant differences between males and females with regard to leukocyte concentrations.

Effect of Health status

The presence of some diseases can also contribute to the occurrence of metabolic changes, more specifically, in the concentrations of some blood metabolites. In this sense, Paiano et al. (2019) using Holstein cows, investigated the effects of the presence of anemia in the concentrations of some serological parameters. The authors concluded that anemic cows had lower red blood cell count as well as lower hemoglobin, hematocrit, serum cholesterol and calcium concentrations. On the other hand, the animals that were affected by this disease had higher white blood cell and platelet counts as well as higher mean crepuscular hemoglobin, non-esterified fatty acids (NEFA), β-hydroxybutyrate, fibrinogen and globulin concentrations.

With the same objective Macdonald et al. (2017) evaluated the impacts caused by liver abscesses. They observed that the presence of abscesses significantly increased cortisol and aspartate aminotransferase contents. In contrast, these animals had significantly lower albumin, cholesterol and testosterone levels. Other parameters evaluated there were slightly higher values of leptin, carbon dioxide, FSH and prolactin in animals with abscesses in the liver. Conversely, the levels of IGF-1, ALP, acetate, glucose, urea were slightly lower in these animals compared to those without abscesses.

Gregory et al. (2004) studied the possible impacts of leukosis virus on urea and creatine concentrations in Jersey animals. They concluded that the presence of this virus has no influence on the concentrations of these 2 metabolites.

Effects of Physiological stage, parity and age at first calving

When we think about the changes in the metabolic profile of animals and the factors that influence those changes, we have to consider some reproductive aspects. These aspects include the physiological stage of the animal (pregnant/not pregnant; lactating, dry, etc.) as well as the parity and the age at first birth.

Pereira et al. (1987) studied the changes in the blood concentrations of some metabolites during pregnancy in Spanish native animals, from the Blanca Cacereña breed. They concluded that as gestation progresses there was an increase in the number of erythrocytes, leukocytes and cell volume. Regarding pregnancy, Wood et al. (2013), using crossbreed animals (Simental x Angus) have assessed how some metabolites behave considering whether the animal was pregnant or not. Pregnant cows had higher levels of β-hydroxybutyrate (BHBA), Non-esterified fatty acids (NEFA) and urea than non-pregnant cows. On the other hand, the cholesterol levels were higher in the non-pregnant animals.

Gonano et al. (2014) studied the blood concentrations variation of some metabolites in cross-beef animals. The authors divided the animals into 3 groups according to their physiological stages: yearling (open), early-gestation and late-gestation. Creatinine, acetate, carbon dioxide contents as well as aspartate aminotransferase activity were higher in open heifers and lower in late-gestation females. Albumin values were higher in the early-gestation cows, followed by the late-gestation and, with the lowest levels, the open ones. Finally, the authors recorded higher urea values and higher enzyme activity of glutamate dehydrogenase in the open animals, followed by the late gestation and the early gestation females were the ones with the lowest values.

In 1981, Petterson & Walden conducted a study in the same pattern as the one mentioned above. Using Holstein females, the two studies divided the animals into 3 groups considering their physiological stage (lactating pregnant; lactating non-pregnant and dry) in order to evaluate the differences in serological concentrations of some metabolites. Starting with minerals, the authors found that for calcium, the group of animals with the highest levels was the group of the dry cows, followed by the lactating nonpregnant. For inorganic phosphorus it is again the non-lactating animals which have the highest values, in this case followed by the lactating pregnant ones. Regarding the glucose and creatinine levels no significant differences were found between the different physiological stages. The blood urea nitrogen (BUN) and the cholesterol contents were higher in the lactating pregnant cows and lower in the dry animals. In the case of uric acid the group of animals with higher concentrations was the lactating pregnant, contrasting with the total proteins where dry animals were the ones with the highest values. Petterson & Walden also observed that pregnant lactating animals had the highest amounts of serological albumin, contrasting with the non-pregnant lactating that had the lowest values. In enzyme terms, the activity of the enzymes LDH and serum glutamic oxaloacetic transaminase (SGOT or AST) was significantly lower in the non-pregnant lactating female group. Finally, alkaline phosphatase activity was higher in dry cows and significantly lower in the pregnant lactating.

In order to study metabolic changes throughout the lactation stage, Doornenbal et al. (1988), conducted a study using Shorthorn cows. In this study, they were able to observe that the highest glucose values were recorded at parturition, with decreasing levels during the lactation phase. The creatinine concentrations decreased during the lactation, followed by an increase during the postweaning. Regarding the urea and uric acid levels these are significantly higher in lactating animals than in non-lactating cows. The authors could also conclude that both LDH and AST enzymes increase their activity during lactation.

Hernández, in his research carried out in 1992 with the Morenas Gallega's breeds (Cachena, Caldelana, Frieiresa, Limiana, Vianesa), in addition to assessing the effects of age on serological concentrations of some metabolites, also studied the impact of different physiological stages on these concentrations. The author considered two different physiological states (gestational animals and lactating animals, although they can co-exist) and evaluated the differences in the metabolic levels. Table 2 and 3 shows the results of Hernández in relation to the hematological values and the leukocyte formula respectively, where it is described in which physiological stage was the highest serological concentration (Gestation or Lactation) of each metabolite in each studied breed.

Table 2 - Comparison of hematological values between lactating and gestating animals.

Metabolite	Cachena	Caldelana	Frieiresa	Limiana	Vianesa
Hemoglobin	Gestation	Gestation	Lactation	Lactation	Lactation
Hematocrit	Lactation	Lactation	Gestation	Gestation	Gestation
Erythrocyte	Gestation	Lactation	Gestation	Gestation	Gestation
Leukocyte	Gestation	Without differences	Gestation	Without differences	Without differences

When "Gestation" arises it means that the concentration of the metabolite in the indicated breed is higher in the animals in pregnancy compared to those in lactation, and vice versa.

** - Statistically significant.*

Source: Hernandez, 1992

Table 3 - Comparison of leukocytary formula values between lactating and gestating animals.

Metabolite	Cachena	Caldelana	Frieiresa	Limiana	Vianesa
Neutrophils	Without differences	Without differences	Without differences	Without differences	Without differences
Lymphocytes	Gestation	Gestation	Gestation	Lactation	Gestation
Eosinophils	Lactation*	Gestation	Lactation	Lactation	Lactation
Monocytes	Gestation*	Gestation	Gestation	Lactation	Lactation*
Basophils	Lactation*	Gestation	Lactation	Gestation*	Gestation

When "Gestation" arises it means that the concentration of the metabolite in the indicated breed is higher in the animals in pregnancy compared to those in lactation, and vice versa.

** - Statistically significant.*

Source: Hernandez, 1992

In addition to these values, the author also evaluated the differences in the activity of some enzymes, shown in Table 4.

Table 4 - Comparison of some enzyme's activity between lactating and gestating animals.

Metabolite	Cachena	Caldelana	Frieiresa	Limiana	Vianesa
AST	Lactation	Lactation	Gestation	Gestation	Lactation
ALT	Gestation	Lactation	Lactation	Lactation	Lactation
PA	Without differences	Gestation*	Without differences	Without differences	Without differences
LDH	Lactation*	Gestation	Gestation	Gestation	Gestation*

When "Gestation" arises it means that the enzyme's activity in the indicated breed is higher in the pregnancy animals compared to those in lactation, and vice versa.

** - Statistically significant.*

Source: Hernandez, 1992

Table 5 shows more results obtained by the author, concerning some blood metabolites.

Table 5 - Comparison of some blood metabolite values between lactating and gestating animals.

Metabolite	Cachena	Caldelana	Frieiresa	Limiana	Vianesa
Glucose	Lactation	Gestation	Lactation*	Gestation	Lactation
Total Lipids	Gestation	Lactation	Lactation	Lactation	Lactation*
Triglyceride	Gestation	Lactation	Lactation	Gestation	Gestation
Cholesterol	Gestation	Gestation	Lactation	Lactation	Lactation
Free Fatty Acids	Lactation	Without differences	Lactation	Lactation	Gestation
Creatinine	Lactation*	Lactation*	Gestation	Gestation	Lactation*
Urea	Lactation*	Gestation	Lactation	Gestation	Lactation
Total Bilirubin	Gestation	Lactation	Lactation	Lactation	Lactation
Total Proteins	Gestation	Without differences	Lactation*	Without differences	Lactation*
Albumin	Lactation	Lactation	Gestation	Lactation	Lactation

When "Gestation" arises it means that the concentration of the metabolite in the indicated breed is higher in the animals in pregnancy compared to those in lactation, and vice versa.

** - Statistically significant.*

Source: Hernandez, 1992

Finally, Hernandez's study included the analysis of the contents of some blood minerals. These results are described in Table 6.

Table 6 - Comparison of some blood minerals concentration between lactating and gestating animals.

Metabolite	Cachena	Caldelana	Frieiresa	Limiana	Vianesa
Calcium	Gestation	Gestation	Lactation	Lactation	Lactation
Phosphorus	Gestation	Gestation	Lactation	Lactation	Lactation*
Magnesium	Gestation	Gestation	Lactation	Lactation	Gestation
Sodium	Gestation	Lactation	Gestation	Gestation	Lactation
Potassium	Gestation*	Gestation	Gestation	Gestation	Gestation
Iron	Lactation*	Gestation	Gestation	Gestation	Lactation
Copper	Gestation	Lactation	Gestation	Gestation*	Gestation*
Zinc	Gestation	Gestation	Lactation	Gestation	Gestation
Manganese	Lactation	Lactation	Gestation	Lactation	Lactation
Cobalt	Gestation	Lactation	Lactation	Lactation	Gestation
Molybdenum	Gestation	Lactation	Lactation	Gestation	Gestation
Selenium	Lactation	Lactation	Lactation	Gestation	Gestation

When "Gestation" arises it means that the concentration of the metabolite in the indicated breed is higher in the animals in pregnancy compared to those in lactation, and vice versa.

** - Statistically significant.*

Source: Hernandez, 1992

In 2011, Quintela et al. evaluated the influence of the number of calving in the concentration of some blood metabolites. With females of the Rubia Gallega breed, the authors concluded that albumin, calcium, phosphorus and magnesium concentrations were higher in heifers compared to multiparous. On the other hand, the multiparous cows had higher values of total proteins when compared with heifers.

Regarding the age at first birth Nyman et al. (2008) conducted a study in primiparous cows. The authors observed that the animals who had their first birth older than 27 months had higher values of BHBA (Beta-Hydroxybutyrate) and NEFA (Non-esterified Fatty Acids), in contrast to the levels of glucose, insulin and nitrogenous urea that were higher in animals that had their first delivery less than 27 months of age. Throughout this study Nyman et al. also verified the metabolic differences in cows whose first delivery occurred when the animals were more (vs less) than 25 months old. They concluded that animals that had their first delivery less than 25 months old had higher blood concentrations of glutamine and nitrogenous urea and lower concentrations of NEFA.

Effects of stress and physical exercise

The physical exercise and the presence of stressful situations can also influence the metabolism of the animals. In order to evaluate the impact of physical exercise on the metabolic concentrations of some blood parameters, Arave et al. (1978) conducted a study using animals

of the Holstein breed. In this study, the authors concluded that glucocorticoid and hemoglobin concentrations increased when animals were subjected to physical exertion. The other metabolites evaluated (packed cell volume, leucocytes, eosinophils, neutrophils, lymphocytes, monocytes and basophils) did not suffer any differences in their concentrations due to physical exertion. With the same objective, Escalera-Valente et al. (2013) verified the impact of physical exercise on animals of the lidia breed. They observed that, in these animals, physical exertion caused an increase in blood pH as well as in the concentrations of HCO_3^- (bicarbonate), PO_2 (oxygen partial pressure) and sO_2 (oxygen saturation). On the other hand, with exercise, the concentrations of PCO_2 (carbon dioxide partial pressure), hemoglobin and lactate have decreased. Sodium, potassium, calcium and hematocrit values remained without significant changes.

Sanchez et al. (1996) evaluated the adaptative responses in females of the Lidia breed submitted to different sequences of stress. They concluded that there was a large increase in cortisol, glucose, ALT and creatine levels in each of the experimental situations. On the other hand the chloride concentrations decreased in animals submitted to stress. Furthermore the authors noticed an increase (not always significant) of uric acid, AST, CK and calcium, and a decrease in triglycerides phosphorus. In relation to either sodium or urea no significant variations were found.

Escalera-Valente et al. (2021a) studied the effect of intense exercise on some blood biochemical variables in Lidia cattle. After undergoing intense physical effort, the animals presented significant increases in the contents of total proteins, albumin, triglycerides, cholesterol, uric acid, creatinine, urea and glucose as well as higher levels of LDH, CK, AST, ALP, GGT, and ALT enzymes and cortisol. Escalera-Valente et al. (2021b) also evaluated the impact of physical exercise on serological concentrations of some minerals in Lidia animals. The authors verified that the physical exercise caused an increase in the levels of all the studied macrominerals (Ca, Mg, P, K and Na) as well as in the levels of Cr, Ni, Cu and Fe. On the other hand, cobalt levels decreased while zinc, selenium and molybdenum levels remained similar.

From the examples stated above, it is clear that blood metabolic profiling in Iberian breeds has seldom been conducted and the majority of the existing data on the bovine blood metabolic data has been obtained in breeds like Holstein or Angus that are much more studied than the Iberian cattle breeds. In fact, and with the exception of a few pioneering studies, the subject has never been addressed in Portuguese or Spanish Iberian cattle. This is entirely understandable, as these animals are bred in remote farms with limited management infrastructures, because they are very difficult to manage, and even the owners do not allow their sampling, as happens for example in the Lidia breed due to the possibility of modifying their behavior during the fight. However, it is of the utmost importance to fill this knowledge gap in order to know the physiology and adequately manage these animals.

3. Material and Methods

3.1. Animal sampling

A total of 311 animals were used in this study. Animals sampled were of the Lidia (Spanish fighting bull), Morucha and Avileña Iberian cattle breeds. Animals were sampled from three different extensive production system farms located in the Montado/Dehesa-type biome during the 2018-2021 period. All farms were located in the Autonomous Community of Castilla y León (NW Spain).

Hematological analyses were conducted in all the 311 animals sampled. However, only 211 were used for biochemical studies. The sampling had the following distribution:

a) 198 (hematological analysis) and 99 (biochemical analysis) from the Lidia breed from a herd of *toros de lidia* located in the province of León;
b) 103 (hematological analysis) and 102 (biochemical analysis) from the Morucha breed from a herd located in the province of Salamanca.
c) 10 (hematological analysis) and 10 (biochemical analysis) from the Avileña-Negra Ibérica breed from a herd located in the province of Salamanca.

Within each breed, there were animals of different ages and sex. This distribution is presented in tables 7 and 8 for animals used for hematological and biochemical analysis, respectively.

Table 7: Distribution of the animals of the different breeds studied (Lidia; Morucha and Avileña-Negra Ibérica) according to sex and age.

	Age							
Breed	**<1 year**		**2 years**		**3 years**		**> 4 years**	
Lidia	♂ 22	♀ 12	♂ 0	♀ 30	♂ 0	♀ 0	♂ 19	♀ 115
Morucha	♂ 0	♀ 3	♂ 0	♀ 12	♂ 0	♀ 9	♂ 0	♀ 79
Avileña	♂ 0	♀ 0	♂ 0	♀ 0	♂ 0	♀ 0	♂ 0	♀ 10
Total	♂ 22	♀ 15	♂ 0	♀ 42	♂ 0	♀ 9	♂ 19	♀ 204

All 311 animals were used for haematological analysis. ♂ - Males ♀ - Females

Table 8: Distribution of the animals of the different breeds studied (Lidia; Morucha and Avileña-Negra Ibérica) according to sex and age

	Age							
Breed	**<1 year**		**2 years**		**3 years**		**> 4 years**	
Lidia	♂ 11	♀ 0	♂ 0	♀ 10	♂ 0	♀ 10	♂ 19	♀ 49
Morucha	♂ 0	♀ 0	♂ 0	♀ 13	♂ 0	♀ 9	♂ 0	♀ 80
Avileña	♂ 0	♀ 0	♂ 0	♀ 0	♂ 0	♀ 0	♂ 0	♀ 10
Total	♂ 11	♀ 0	♂ 0	♀ 23	♂ 0	♀ 19	♂ 19	♀ 139

These animals (n=211) were used for biochemical analysis. ♂ - Males ♀ - Females

3.2. Sample collection and processing

Although blood collection using a large gauge syringe and needle is usually recommended to avoid hemolysis, in our case blood samples were collected using two vacuum tubes (one with heparin (10 ml) and the other with EDTA (10 ml). Blood was sampled from the coccygea (caudal) vein. Animals were restrained in a cattle handling crush (squeeze chute), following standard veterinary procedures in commercial cattle production in the region. Samples were obtained during standard routine sampling and conducted by veterinarians certified by the Consejo General de Colegios Veterinarios de España (Madrid, Spain). As this collection was part of a standard herd health practices locally conducted in the framework of regular veterinary work, as Official Livestock Sanitation Campaigns (*Campañas Oficiales de Saneamento Ganadero)* that furthermore involved no animal experimentation, no ethics committee permit was thus deemed necessary.

The EDTA containing tube, after being thoroughly homogenized, was used for haematological measurements. They were carried out with an Abacus Junior Vet CVM analyser (Diatron, Budapest, Hungary), shown in figure 23 and following manufacturer's instructions.

Measured haematological parameters include including red blood cells (RBCs) and white blood cells (WBCs) count and hemoglobin concentration (Hb), packed cell volume or hematocrit (PCV or Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean platelet volume (PLQ).

Figure 23: Abacus Junior Vet CVM analyzer
Source: J.Ramiro Gonzalez, 2021

The heparin tube was used to obtain plasma. For this, the blood with heparin was centrifuged at 4000 rpm (2200 x g) for 15 minutes in a standard benchtop centrifuge. After centrifugation, the plasma was collected free of impurities with a 1000 mL micropipette and placed in Eppendorf tubes, figure 24, and subsequently stored at -20 ºC until further analysis in the Laboratorio de Técnicas Instrumentales (L.T.I.) of the University of León. From plasma analysis several biochemical parameters, as well as several different minerals were measured.

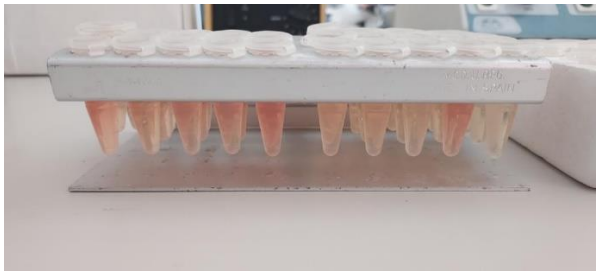

Figure 24: Eppendorf tubes containing plasma free of impurities after the centrifugation
Source: J.Ramiro Gonzalez, 2021

To determinate the biochemical parameters, a BA 400 Biosystems autoanalyzer (Biosystems, Barcelona, Spain) was used employing Roche Diagnostic reagents and following manufacturer's instructions. The biochemical variables measured were total proteins (TP), albumin (Alb), glucose (Glu), blood urea nitrogen (BUN), creatinine (Cre), enzymes (alkaline phosphatase [ALP], gamma-glutamyl transferase [GGT], aspartate aminotransferase [AST, formerly GOT], alanine aminotransferase [ALT, formerly GPT], creatine kinase [CK]), as wells as the macrominerals calcium (Ca), phosphorus (P), magnesium (Mg).

The analyzed trace elements were: selenium (Se), cooper (Cu), iron (Fe), zinc (Zn). The samples were analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES)

or by inductively coupled plasma mass spectrometry (ICP-MS) as presented in figures 25 and 26 respectively, depending on the amount of each mineral present in the sample.

Figure 25: Plasma atomic emission spectrometry (ICP-AES)
Source: J.Ramiro Gonzalez, 2021

Figure 26: Inductively coupled plasma mass spectrometry (ICP-MS)
Source: J.Ramiro Gonzalez, 2021

Samples were analyzed using a ICP-AES (Optima 2000DV, PerkinElmer Instruments, Waltham, MA, USA) needed addition of 5 ppm Sc, used as an internal standard. The detection limit was 0.1 ppm (mg/kg). However, the samples analysed by ICP-MS (Varian Iberica; Madrid, Spain), allow the measurement of elements in a liquid matrix with sensitivity below 0.2 ppb (µg/kg). The samples were diluted 1:10 in a solution of 0.05% EDTA and 0.5% nitric acid to which 10 ppb of a solution mixture of elements used as internal standard was added.The blanks and standards were prepared using the technique of additions on bovine plasma also diluted 1:10 in the previously mentioned EDTA-nitric solution. To check the accuracy of the analytical method, a multielement standard solution (Merck, Temecula, CA, USA) with different concentrations (0, 10, 50 and 100 ppb) was used for calibration.

3.3. Statistical analysis

Data was analyzed using the ANOVA-single factor procedure of the Analysis toolpak of Microsoft Excel. Significance was declared when $p<0.05$, The following comparisons were made:

- Hematological: Avileña vs Lidia vs Morucha; Avileña Females vs Lidia Females vs Morucha Females; Lidia: Males vs Females; Lidia Yearlings: Males vs Females; Lidia Males: Yearlings vs Adults; Lidia Females: Yearlings vs 2 years old vs Adults; Morucha Females: Yearlings vs 2 years old vs 3 years old vs Adults.

- Biochemical: Avileña vs Lidia vs Morucha; Avileña Females vs Lidia Females vs Morucha Females; Lidia: Males vs Females; Lidia Males: Yearlings vs Adults; Lidia Females: 2 years old vs 3 years old vs Adults; Morucha Females: 2/3 years old vs Adults.

3.4. Disclaimer

Members of the research group of the Veterinary Medicine Unit of the University of Léon, as well as other veterinarians, who worked in collaborations with this Unit, performed most of the sampling, whereas technicians from the Laboratorio de Técnicas Instrumentales (L.T.I.) of the University of León conducted most of the analysis. The initial idea of this dissertation was that the author could do sample collection and processing of additional samples. However, due to the COVID-19 pandemic confinements during the Winter and Spring of 2021, the number of collected and analyzed samples had to be substantially reduced and thus limited to archived samples. It must be finally stated that the author conducted sampling and analysis of a limited number of samples, as well as the entire statistical analysis and results interpretation.

4.Results

The results obtained for the different comparisons conducted are presented in this section. They are divided according to the type of analysis: hematological or biochemical.

4.1. Hematological results

Regarding the hematological results, these are presented in tables 9, 10, 11, 12, 13, 14 and 15. The hematological results considering the breed of the animals (Avileña, Lidia and Morucha) are shown in tables 9 and 10. Table 10 covers all animals while table 9 only compares the females of the different breeds.

Table 9 : Descriptive statistics of all animals sampled of some hematological parameters.

Parameter	Breed	n	Mean	SD	Minimum	Maximum	R. C. V	p-value
WBC **10⁶*	Avileña	10	7.44	1.01	5.93	9.45	4 -12	Av x Lid - 0.114
	Lidia	183	10.18	5.17	2.60	57.40		Lid x Mor – 0.173
	Morucha	103	11.42	9.98	3.69	58.30		Mor x Av – 0.211
RBC **10⁶*	Avileña	10	6.62	1.03	8.13	5.12	5 – 10	Av x Lid – 0.624
	Lidia	196	6.83	1.34	2.70	11.24		Lid x Mor – 0.177
	Morucha	103	7.03	1.07	4.84	11.15		Mor x Av - 0.242
Hb *g/L*	Avileña	10	12.56 [a c]	1.55	10.30	16.00	8 - 15	Av x Lid – 0.475
	Lidia	198	13.11 [b c]	2.39	3.90	20.90		Lid x Mor – 0.004
	Morucha	103	12.34 [a]	1.72	8.90	17.00		Mor x Av – 0.701
Ht *%*	Avileña	10	37.87 [a c]	5.17	29.00	48.20	24 – 46	Av x Lid – 0.713
	Lidia	197	36.94 [b c]	7.99	9.90	68.90		Lid x Mor – 0.002
	Morucha	103	39.89 [a]	7.08	25.10	57.84		Mor x Av – 0.381
MCV *fL*	Avileña	10	51.07 [a]	4.00	43.80	56.80	40 – 60	Av x Lid – 0.0548
	Lidia	198	54.50 [a]	5.50	41.50	74.00		Lid x Mor – 0.01
	Morucha	103	56.57 [b]	8.22	9.10	77.00		Mor x Av – 0.039
MCH *pg*	Avileña	10	16.96 [a]	1.15	14.60	18.20	11 – 17	Av x Lid – 5.54E-05
	Lidia	197	19.44 [b]	1.90	8.10	27.80		Lid x Mor - 4,17E-16
	Morucha	103	17.64 [a]	1.34	13.90	21.10		Mor x Av – 0.124
MCHC *g/L*	Avileña	10	33.23 [a]	1.00	31.90	35.50	30 – 36	Av x Lid – 0.028
	Lidia	197	35.91 [b]	3.81	15.50	47.70		Lid x Mor – 3.88E-25
	Morucha	103	31.23 [c]	2.36	25.40	36.30		Mor x Av – 0.009
PLQ ***10⁹/L***	Avileña	10	186.10 [a c]	93.65	21.00	314.00	-	Av x Lid – 0.116
	Lidia	183	148.74 [b c]	71.60	18.00	385.00		Lid x Mor – 7.34E18
	Morucha	102	248.01 [a]	109.98	17.00	698.00		Mor x Av – 0.088

The parameters studied are: WBC (white blood cells); RBC (red blood cells); Hb (hemoglobin); Ht (hematocrit); MCV (mean corpuscular volume); MCH (mean corpuscular hemoglobin); MCHC (mean corpuscular hemoglobin concentration) and PLQ (platelets). Different letters in each parameter indicate significant media differences ($p < 0.05$). R. C V indicates the Reference Cattle Values according to Radostits et al., 2006.

Table 10 : Descriptive statistics of all females sampled of some hematological parameters.

Parameter	Females	n	Mean	SD	Minimum	Maximum	R. C. V	p-value
WBC **10⁶*	Avileña	10	7.44 [a c]	1.01	5.93	9.45	4 -12	Av x Lid: 0.100
	Lidia	142	9.29 [b c]	3.52	2.60	20.50		Lid x Mor: 0.019
	Morucha	103	11.42 [a]	9.98	3.69	58.30		Mor x Av: 0.211
RBC **10⁶*	Avileña	10	6.62 [a c]	1.03	8.13	5.12	5 – 10	Av x Lid: 0.972
	Lidia	156	6.63 [b c]	1.30	2.70	11.24		Lid x Mor: 0.010
	Morucha	103	7.03 [a]	1.07	4.84	11.15		Mor x Av: 0.242
Hb *g/L*	Avileña	10	12.56	1.55	10.30	16.00	8 - 15	Av x Lid: 0.678
	Lidia	157	12.88	2.40	3.90	20.90		Lid x Mor: 0.050
	Morucha	103	12.34	1.72	8.90	17.00		Mor x Av: 0.053
Ht *%*	Avileña	10	37.87 [a c]	5.17	29.00	48.20	24 – 46	Av x Lid: 0.380
	Lidia	157	35.76 [b c]	7.47	9.90	68.90		Lid x Mor:1.23E05
	Morucha	103	39.89 [a]	7.08	25.10	57.84		Mor x Av: 0.381
MCV *fL*	Avileña	10	51.07 [a]	4.00	43.80	56.80	40 – 60	Av x Lid - 0.058
	Lidia	157	54.37 [a]	5.38	41.50	74.00		Lid x Mor: 0.010
	Morucha	103	56.57 [b]	8.22	9.10	77.00		Mor x Av: 0.039
MCH *pg*	Avileña	10	16.96 [a]	1.15	14.60	18.20	11 – 17	Av x Lid: 2.2E-06
	Lidia	157	19.65 [b]	1.71	15.50	27.80		Lid x Mor: 2.2E20
	Morucha	103	17.64 [a]	1.34	13.90	21.10		Mor x Av: 0.123
MCHC *g/L*	Avileña	10	33.23 [a]	1.00	31.90	35.50	30 – 36	Av x Lid: 0.004
	Lidia	157	36.35 [b]	3.43	27.30	46.30		Lid x Mor: 6.4E-31
	Morucha	103	31.23 [c]	2.36	25.40	36.30		Mor x Av: 0.009
PLQ ***10⁹/L**	Avileña	10	186.10 [a c]	93.65	21.00	314.00	-	Av x Lid: 0.160
	Lidia	149	152.14 [b c]	72.17	18.00	385.00		Lid x Mor: 4.9E-15
	Morucha	102	248.01 [a]	109.98	17.00	698.00		Mor x Av: 0.088

The parameters studied are: WBC (white blood cells); RBC (red blood cells); Hb (hemoglobin); Ht (hematocrit); MCV (mean corpuscular volume); MCH (mean corpuscular hemoglobin); MCHC (mean corpuscular hemoglobin concentration) and PLQ (platelets). Different letters in each parameter indicate significant media differences ($p < 0.05$). R. C V indicates the Reference Cattle Values according to Radostits et al., 2006.

The hematological values according to the sex of the animals are shown in tables 11 and 12. The first one compares males, females from the Lidia breed while in table 12 only the yearling males, and females (from the Lidia breed) are compared.

Table 11 : Descriptive statistics of Lidia males and females of some hematological parameters.

Parameter	Males/Females	n	Mean	SD	Minimum	Maximum	R. C .V	p-value
WBC **10⁶*	Males	41	13.26 [a]	9.06	4.00	57.40	4 – 12	2.64E-05
	Females	142	9.29 [b]	3.52	2.60	20.52		
RBC **10⁶*	Males	40	7.59 [a]	1.29	5.01	10.97	5 – 10	3.68E-05
	Females	156	6.63 [b]	1.30	2.70	11.24		
Hb *g/L*	Males	41	13.98 [a]	2.18	8.90	20.40	8 – 15	0,0082
	Females	157	12.88 [b]	2.40	3.90	20.90		
Ht *%*	Males	40	41.59 [a]	8.38	25.30	66.30	24 – 46	2,03E-05
	Females	157	35.76 [b]	7.47	9.90	68.90		
MCV *fL*	Males	41	54.99	5.89	42.90	66.40	40 – 60	0,5308
	Females	157	54.37	5.38	41.50	74.00		
MCH *pg*	Males	39	18.64 [a]	2.48	8.10	21.90	11 – 17	0,0026
	Females	157	19.65 [b]	1.71	15.50	27.80		
MCHC *g/L*	Males	40	34.19 [a]	4.72	15.50	47.70	30 – 36	0,0012
	Females	157	36.35 [b]	3.43	27.30	46.30		
PLQ ***10⁹/L**	Males	34	133.82	68.08	65.00	377.00	-	0,1790
	Females	149	152.14	72.17	18.00	385.00		

The parameters studied are: WBC (white blood cells); RBC (red blood cells); Hb (hemoglobin); Ht (hematocrit); MCV (mean corpuscular volume); MCH (mean corpuscular hemoglobin); MCHC (mean corpuscular hemoglobin concentration) and PLQ (platelets). Different letters in each parameter indicate significant media differences ($p < 0.05$) R. C V indicates the Reference Cattle Values according to Radostits et al., 2006.

Table 12 : Descriptive statistics of yearling Lidia males and females of some hematological parameters.

Parameter	Yearling Males/Females	n	Mean	SD	Minimum	Maximum	R. C .V	p-value
WBC **10⁶*	Males	22	10.50 [a]	4.49	4.00	21.10	4 – 12	0,0445
	Females	11	7.57 [b]	1.42	3.70	9.60		
RBC **10⁶*	Males	21	7.38	1.25	5.01	10.97	5 – 10	0,8393
	Females	12	7.46	0.81	5.60	9.07		
Hb *g/L*	Males	22	13.35	2.06	8.90	16.10	8 – 15	0,0669
	Females	12	14.67	1.66	11.80	17.50		
Ht *%*	Males	21	39.68	7.61	25.30	57.30	24 – 46	0,7933
	Females	12	39.04	4.53	30.80	48.70		
MCV *fL*	Males	22	54.05	5.98	42.90	63.40	40 – 60	0,3492
	Females	12	52.34	2.17	59.40	48.20		
MCH *pg*	Males	21	18.40	2.84	8.10	21.90	11 – 17	0,1520
	Females	12	19.65	0.96	18.10	21.80		
MCHC *g/L*	Males	21	34.42	5.72	15.50	47.70	30 – 36	0,0697
	Females	12	37.58	0.97	35.20	39.20		
PLQ ***10⁹/L**	Males	15	121.80 [a]	56.85	74.00	269.00	-	0,0387
	Females	12	181.58 [b]	85.22	39.00	314.00		

The parameters studied are: WBC (white blood cells); RBC (red blood cells); Hb (hemoglobin); Ht (hematocrit); MCV (mean corpuscular volume); MCH (mean corpuscular hemoglobin); MCHC (mean corpuscular hemoglobin concentration) and PLQ (platelets). Different letters in each parameter indicate significant media differences ($p < 0.05$). R. C V indicates the Reference Cattle Values according to Radostits et al., 2006.

Tables 13, 14 and 15 show the hematological results of the animals according to age. Lidia male's results are analyzed in the first, Lidia females in the second and Morucha females in the third.

Table 13: Descriptive statistics of Lidia yearling and adult males of some hematological parameters.

Parameter	Lidia Males	n	Mean	SD	Minimum	Maximum	R. C .V	p-value
WBC **10⁶*	Yearlings	22	10.50 [a]	4.49	4.00	21.10	4 – 12	0,0298
	Adults	19	16.47 [b]	13.92	6.00	57.40		
RBC **10⁶*	Yearlings	21	7.38	1.25	5.01	10.97	5 – 10	0,2478
	Adults	19	7.83	1.38	6.23	10.32		
Hb *g/L*	Yearlings	22	13.35 [a]	2.06	8.90	16.10	8 – 15	0,0440
	Adults	19	14.72 [b]	2.13	12.50	20.40		
Ht *%*	Yearlings	21	39.68	7.61	25.30	57.30	24 – 46	0,1053
	Adults	19	43.69	8.16	38.10	66.30		
MCV *fL*	Yearlings	22	54.05	5.98	42.90	63.40	40 – 60	0,3015
	Adults	19	56.07	3.19	54.60	66.40		
MCH *pg*	Yearlings	21	18.40	2.84	8.10	21.90	11 – 17	0,5639
	Adults	19	18.83	1.69	16.10	21.80		
MCHC *g/L*	Yearlings	21	34.42	5.72	15.50	47.70	30 – 36	0,7468
	Adults	19	33.93	3.44	29.10	38.60		
PLQ *10⁹/L	Yearlings	15	121.80	56.85	74.00	269.00	-	0,3682
	Adults	19	143.32	75.95	65.00	377.00		

The parameters studied are: WBC (white blood cells); RBC (red blood cells); Hb (hemoglobin); Ht (hematocrit); MCV (mean corpuscular volume); MCH (mean corpuscular hemoglobin); MCHC (mean corpuscular hemoglobin concentration) and PLQ (platelets). Different letters in each parameter indicate significant media differences ($p < 0.05$). R. C V indicates the Reference Cattle Values according to Radostits et al., 2006.

Table 14: Descriptive statistics of Lidia yearling, 2 years and adult females of some hematological parameters.

Parameter	Lidia Females	n	Mean	SD	Minimum	Maximum	R. C. V	p-value
WBC **10⁶*	Yearlings	11	7.57	1.42	3.70	9.60	4 -12	Y x 2: 0.327
	2 years	26	8.91	4.32	2.60	18.52		2 x A: 0.412
	Adults	105	9.56	3.42	3.0	20.50		A x Y:0.059
RBC **10⁶*	Yearlings	12	7.46 [a]	0.81	5.60	9.07	5 – 10	Y x 2: 0.154
	2 years	30	6.66 [a c]	1.82	2.70	10.30		2 x A: 0.638
	Adults	114	6.54 [b c]	1.15	3.20	11.20		A x Y: 0.008
Hb *g/L*	Yearlings	12	14.67 [a]	1.66	11.80	17.50	8 - 15	Y x 2: 0.007
	2 years	30	11.94 [b]	3.13	5.50	19.00		2 x A: 0.042
	Adults	115	12.93 [c]	2.13	3.90	20.90		A x Y: 0.007
Ht *%*	Yearlings	12	39.04 [a]	4.53	30.80	48.70	24 – 46	Y x 2: 0.018
	2 years	30	32.21 [b]	9.06	12.70	50.30		2 x A: 0.008
	Adults	115	36.34 [a]	6.97	9.90	68.90		A x Y: 0.192
MCV *fL*	Yearlings	12	52.34 [a]	2.17	59.40	48.20	40 – 60	Y x 2: 2.18E-05
	2 years	30	48.22 [b]	2.63	41.50	53.10		2 x A: 9.51E-15
	Adults	115	56.19 [c]	4.86	46.10	74.00		A x Y: 0.008
MCH *pg*	Yearlings	12	19.65 [a]	0.96	18.10	21.80	11 – 17	Y x 2: 9.05E-05
	2 years	30	17.95 [b]	1.35	15.50	21.60		2 x A: 5.42E-09
	Adults	115	20.06 [a]	1.61	16.10	27.80		A x Y: 0.387
MCHC *g/L*	Yearlings	12	37.58 [a c]	0.97	35.20	39.20	30 – 36	Y x 2: 0.933
	2 years	30	37.51 [a]	2.70	34.10	46.30		2 x A: 0.029
	Adults	115	35.93 [b c]	3.67	27.30	45.60		A x Y: 0.124
PLQ ***10⁹/L**	Yearlings	12	181.58 [a c]	85.22	39.00	314.00	-	Y x 2: 0.892
	2 years	28	177.54 [a]	86.03	63.00	385.00		2 x A: 0.018
	Adults	109	142.38 [b c]	64.71	18.00	375.00		A x Y: 0.056

The parameters studied are: WBC (white blood cells); RBC (red blood cells); Hb (hemoglobin); Ht (hematocrit); MCV (mean corpuscular volume); MCH (mean corpuscular hemoglobin); MCHC (mean corpuscular hemoglobin concentration) and PLQ (platelets). Different letters in each parameter indicate significant media differences ($p < 0.05$). R. C V indicates the Reference Cattle Values according to Radostits et al., 2006.

Table 15: Descriptive statistics of Morucha yearling, 2 year, 3 years and adult females of some hematological parameters

Parameter	Morucha Females	n	Mean	SD	Minimum	Maximum	R. C. V.	p-value
WBC **10⁶*	Yearlings	3	9.46	3.74	6.80	13.73	4 – 12	Yx2: 0.44 | Yx3: 0.31
	2 years	12	8.34	1.77	3.98	10.74		YxA: 0.66 | 2x3: 0.72
	3 years	9	8.10	1.06	6.07	9.73		2xA: 0.22
	Adults	79	12.34	11.21	3.69	58.30		3xA: 0.26
RBC **10⁶*	Yearlings	3	6.74 [a c]	0.65	6.03	7.30	5 – 10	Yx2: 0.16 | Yx3: 0.14
	2 years	12	7.98 [b c]	1.38	5.28	9.92		YxA: 0.87 | 2x3: 0.5
	3 years	9	7.63 [b c]	0.86	6.78	9.26		2xA: 4.9E-04
	Adults	79	6.83 [a]	0.96	4.84	11.15		3xA: 0.02
Hb *g/L*	Yearlings	3	13.33 [a c]	1.65	11.70	15.00	8 – 15	Yx2: 0.67 | Yx3: 0.67
	2 years	12	14.00 [b c]	2.49	9.10	17.00		YxA: 0.12 | 2x3: 0.24
	3 years	9	12.86 [b c]	1.59	11.00	15.10		2xA: 11E-04
	Adults	79	11.99 [a]	1.43	8.90	16.20		3xA: 0.09
Ht *%*	Yearlings	3	44.95 [a]	1.34	43.49	46.13	24 – 46	Yx2: 0.67 | Yx3: 0.94
	2 years	12	47.44 [a]	9.72	26.40	57.84		YxA: 0.04 | 2x3: 0.53
	3 years	9	45.17 [a]	4.71	39.69	50.50		2xA: 6.2E-06
	Adults	79	37.95 [b]	5.75	25.10	52.40		3xA: 4.9E-04
MCV *fL*	Yearlings	3	67.67 [a]	8.62	60.00	77.00	40 – 60	Yx2: 0.009 | Yx3: 0.03
	2 years	12	57.78 [b]	4.04	50.00	64.00		YxA: 0.02 | 2x3: 0.34
	3 years	9	59.44 [b]	3.64	55.00	67.00		2xA: 0.41
	Adults	79	55.64 [b]	8.72	9.10	76.00		3xA: 0.2
MCH *pg*	Yearlings	3	19.80 [a]	0.70	19.30	20.60	11 – 17	Yx2: 7.5E-05 | Yx3: 0.001
	2 years	12	17.18 [b]	0.72	16.30	18.90		YxA: 0.011 | 2x3: 0.43
	3 years	9	16.88 [b]	1.01	15.50	18.70		2xA: 0.19
	Adults	79	17.71 [b]	1.37	13.90	21.10		3xA: 0.079
MCHC *g/L*	Yearlings	3	29.83 [a c]	4.61	25.40	34.60	30 – 36	Yx2: 1 | Yx3: 0.39
	2 years	12	29.83 [b c]	2.16	27.40	34.50		YxA: 0.129 | 2x3: 0.097
	3 years	9	28.47 [b c]	1.04	27.10	29.90		2xA: 0.003
	Adults	79	31.81 [a]	2.09	27.40	36.30		3xA: 9.5E-06
PLQ ***10⁹/L**	Yearlings	2	219.00 [a]	65.05	173.00	265.00	-	Yx2: 0.28 | Yx3: 0.92
	2 years	12	370.92 [a]	184.30	75.00	698.00		YxA: 0.82 | 2x3: 0.06
	3 years	9	228.67 [a c]	120.34	17.00	368.00		2xA: 2.4E-05
	Adults	79	232.38 [b c]	81.84	51.00	463.00		3xA: 0.85

The parameters studied are: WBC (white blood cells); RBC (red blood cells); Hb (hemoglobin); Ht (hematocrit); MCV (mean corpuscular volume); MCH (mean corpuscular hemoglobin); MCHC (mean corpuscular hemoglobin concentration) and PLQ (platelets). Different letters in each parameter indicate significant media differences ($p < 0.05$). R. C V indicates the Reference Cattle Values according to Radostits et al., 2006.

4.2 Biochemical results

Regarding the biochemical results, they are presented in tables 16, 17, 18, 19, 20 and 21. Tables 16 and 17 correspond to the results associated to the animals from different breeds (Avileña, Lidia and Morucha). Table 16 covers all animals while table 17 only compares the females from the different breeds.

Table 16 : Descriptive statistics of all animals sampled of some biochemical parameters.

Parameter	Breed	n	Mean	SD	Minimum	Maximum	R. C. V	p-value
Total Proteins *g/L*	Avileña	10	79.63 [a]	3.92	73.62	86.87	57 – 81	Av x Lid: 7.5E-11
	Lidia	97	62.33 [b]	7.37	48.52	81.10		Lid x Mor: 1E-29
	Morucha	102	74.88 [c]	5.63	62.60	92.80		Mor x Av: 0.011
Albumins *g/L*	Avileña	10	45.08 [a]	4.73	36.78	51.07	21 - 36	Av x Lid: 2E-06
	Lidia	99	37.78 [b]	4.29	22.75	44.92		Lid x Mor: 0.008
	Morucha	101	39.78 [c]	6.07	17.06	60.57		Mor x Av: 0.009
Glucose *mg/dL*	Avileña	10	107.82 [a c]	17.83	82.11	137.94	45 - 76	Av x Lid: 0.211
	Lidia	99	121.11 [a]	32.58	77.65	214.45		Lid x Mor: 1.2E-10
	Morucha	100	88.24 [b c]	35.12	16.95	219.17		Mor x Av: 0.087
Cholesterol *mg/dL*	Avileña	-	-	-	-	-	65 - 220	Av x Lid: -
	Lidia	40	115.72 [a]	53.87	29.61	209.59		Lid x Mor: 1.9E-11
	Morucha	69	179.45 [b]	38.50	119.98	302.10		Mor x Av: -
Creatinine *mg/dL*	Avileña	10	1.50 [a]	0.15	1.29	1.85	1 – 2	Av x Lid: 0.002
	Lidia	99	1.82 [b]	0.31	1.23	2.73		Lid x Mor: 4.1E-06
	Morucha	102	2.11 [c]	0.53	1.18	3.59		Mor x Av: 0.0005
Urea/BUN *mg/dL*	Avileña	10	26.39 [a]	4.70	18.96	37.18	6 - 28	Av x Lid: 0.026
	Lidia	98	20.23 [b]	8.42	7.90	49.62		Lid x Mor: 7.2E-06
	Morucha	102	15.17 [c]	6.96	3.80	37.45		Mor x Av: 2.8E-06
AST *U/L*	Avileña	10	87.29 [a]	14.45	61.96	110.34	78 - 132	Av x Lid: 0.0017
	Lidia	29	217.82 [b]	118.52	75.99	579.47		Lid x Mor: 1.3E-13
	Morucha	97	108.72 [c]	26.56	64.61	215.39		Mor x Av: 0.014
ALT *U/L*	Avileña	10	43.59	7.82	33.10	59.18	11 - 40	Av x Lid: 0.226
	Lidia	99	40.23	8.27	22.02	68.62		Lid x Mor: 0.340
	Morucha	98	43.87	36.72	14.59	365.37		Mor x Av: 0.98
GGT *U/L*	Avileña	9	18.62 [a]	6.10	5.53	24.19	6.1 – 17.4	Av x Lid: 0.090
	Lidia	99	37.07 [a]	35.27	0.29	308.10		Lid x Mor: 1.4E-10
	Morucha	85	10.97 [b]	7.01	0.10	36.90		Mor x Av: 0.016
ALP *U/L*	Avileña	10	98.40 [a c]	91.40	34.94	332.44	0 – 500	Av x Lid: 0.467
	Lidia	98	122.47 [a]	99.11	28.94	710.51		Lid x Mor: 7.6E-04
	Morucha	98	75.89 [b c]	90.32	17.19	594.65		Mor x Av: 0.459
CK *U/L*	Avileña	10	318.32	244.68	147.12	1036	35 - 280	Av x Lid: 0.288
	Lidia	21	472.23	400.32	130.29	1651		Lid x Mor: 0.707
	Morucha	56	432.62	406.45	82.05	2365		Mor x Av: 0.399
Ca *mg/dL*	Avileña	10	10.02 [a]	1.21	8.52	13.03	9.7 – 12.4	Av x Lid: 0.001
	Lidia	99	9.24 [b]	0.61	7.25	10.63		Lid x Mor: 1E-08
	Morucha	102	9.97 [a]	1.04	8.12	13.20		Mor x Av: 0.884
P *mg/dL*	Avileña	10	7.22 [a]	1.24	5.32	9.20	5.6 – 6.5	Av x Lid: 2.2E-08
	Lidia	99	4.73 [b]	1.23	2.11	7.75		Lid x Mor: 1.2E-11
	Morucha	102	6.00 [c]	1.27	3.49	10.37		Mor x Av: 0.004
Mg *mg/dL*	Avileña	10	2.60 [a]	0.25	2.29	2.99	1.8 – 2.3	Av x Lid: 2.6E-04
	Lidia	99	2.22 [b c]	0.30	1.45	2.93		Lid x Mor: 0.49
	Morucha	102	2.28 [a c]	0.76	1.20	6.97		Mor x Av: 0.189
Cu *ug/dL*	Avileña	10	798.56 [a]	125.10	568.40	1054	157	Av x Lid: 0.017
	Lidia	97	578.23 [b c]	282.73	44.43	1199		Lid x Mor: 0.09
	Morucha	102	656.18 [a c]	359.92	55.73	1208		Mor x Av: 0.212
Fe *ug/dL*	Avileña	10	165.64	27.95	135.79	233.89	100 - 290	Av x Lid: 0.48
	Lidia	99	154.90	46.60	69.77	330.37		Lid x Mor: 0.77
	Morucha	102	158.96	129.88	67.75	1035		Mor x Av: 0.872
Zn *ug/dL*	Avileña	10	962.78 [a]	90.39	837.60	1140	232	Av x Lid: 2.3E-11
	Lidia	89	191.78 [b]	317.75	8.91	1141		Lid x Mor: 1.9E-06
	Morucha	102	466.90 [c]	433.45	5.09	1333		Mor x Av: 5.2E-04
Se *ug/dL*	Avileña	10	34.78 [a]	13.63	20.00	61.7	-	Av x Lid: 0.009
	Lidia	89	64.77 [b]	34.93	23.84	226.98		Lid x Mor: 9.2E-05
	Morucha	73	46.89 [c]	16.01	15.80	81.54		Mor x Av: 0.027

The parameters studied are: total proteins; albumins; glucose; cholesterol; creatinine; Urea/BUN; AST (aspartate aminotransferase); ALT (alanine aminotransferase); GGT (gamma-glutamyl transferase); ALP (alkaline phosphatase); CK (creatine kinase); Calcium; Phosphorus; Magnesium; Copper; Iron; Zinc; Selenium. Different letters in each parameter indicate significant media differences ($p < 0.05$). R. C V indicates the Reference Cattle Values according to Radostits et al., 2006.

Table 17 : Descriptive statistics of all females sampled of some biochemical parameters.

Parameter	Females	n	Mean	SD	Minimum	Maximum	R. C . V	p-value
Total Proteins *g/L*	Avileña	10	79.63 [a]	3.92	73.62	86.87	57 – 81	Av x Lid: 2E-16
	Lidia	67	60.52 [b]	5.46	48.52	74.91		Lid x Mor: 2.2E-36
	Morucha	102	74.88 [c]	5.63	62.60	92.80		Mor x Av: 0.011
Albumins *g/L*	Avileña	10	45.08 [a]	4.73	36.78	51.07	21 - 36	Av x Lid: 4.9E-06
	Lidia	69	38.34 [b]	3.89	26.33	44.92		Lid x Mor: 0.086
	Morucha	101	39.78 [b]	6.07	17.06	60.57		Mor x Av: 0.009
Glucose *mg/dL*	Avileña	10	107.82 [a c]	17.83	82.11	137.94	45 - 76	Av x Lid: 0.67
	Lidia	69	111.65 [a]	26.99	77.65	214.45		Lid x Mor: 7E-06
	Morucha	100	88.24 [b c]	35.12	16.95	219.17		Mor x Av: 0.087
Cholesterol *mg/dL*	Avileña	-	-	-	-	-	65 - 220	Av x Lid: -
	Lidia	14	157.70	34.66	108.90	209.59		Lid x Mor: 0.056
	Morucha	69	179.45	38.50	119.98	302.10		Mor x Av: -
Creatinine *mg/dL*	Avileña	10	1.50 [a]	0.15	1.29	1.85	1 – 2	Av x Lid: 0.003
	Lidia	69	1.80 [b]	0.29	1.29	2.64		Lid x Mor: 1.4E-05
	Morucha	102	2.11 [c]	0.53	1.18	3.59		Mor x Av: 5.2E-04
Urea/BUN *mg/dL*	Avileña	10	26.39 [a]	4.70	18.96	37.18	6 - 28	Av x Lid: 0.015
	Lidia	68	19.31 [b]	8.74	7.90	49.62		Lid x Mor: 8.5E-04
	Morucha	102	15.17 [c]	6.96	3.80	37.45		Mor x Av: 2.8E-06
AST *U/L*	Avileña	10	87.29 [a]	14.45	61.96	110.34	78 - 132	Av x Lid: 1.6E-04
	Lidia	14	140.05 [b]	32.96	75.99	205.79		Lid x Mor: 1.4E-04
	Morucha	97	108.72 [c]	26.56	64.61	215.39		Mor x Av: 0.014
ALT *U/L*	Avileña	10	43.59	7.82	33.10	59.18	11 - 40	Av x Lid: 0.257
	Lidia	69	40.33	8.40	22.02	68.62		Lid x Mor: 0.435
	Morucha	98	43.87	36.72	14.59	365.37		Mor x Av: 0.981
GGT *U/L*	Avileña	9	18.62 [a]	6.10	5.53	24.19	6.1 – 17.4	Av x Lid: 0.11
	Lidia	69	38.53 [a]	39.83	0.29	308.10		Lid x Mor: 2.5E-09
	Morucha	85	10.97 [b]	7.01	0.10	36.90		Mor x Av: 0.016
ALP *U/L*	Avileña	10	98.40 [a c]	91.40	34.94	332.44	0 – 500	Av x Lid: 0.644
	Lidia	68	116.14 [a]	114.17	28.44	710.51		Lid x Mor: 0.013
	Morucha	98	75.89 [b c]	90.32	17.19	594.65		Mor x Av: 0.46
CK *U/L*	Avileña	10	318.32	244.68	147.12	1036	35 - 280	Av x Lid: 0.94
	Lidia	33	477.17	473.64	130.29	1651		Lid x Mor: 0.155
	Morucha	56	432.62	406.45	82.05	2365		Mor x Av: 0.399
Ca *mg/dL*	Avileña	10	10.02 [a]	1.21	8.52	13.03	9.7 – 12.4	Av x Lid: 0.002
	Lidia	69	9.31 [b]	0.53	8.17	10.63		Lid x Mor:3.5E-06
	Morucha	102	9.97 [a]	1.04	8.12	13.20		Mor x Av: 0.88
P *mg/dL*	Avileña	10	7.22 [a]	1.24	5.32	9.20	5.6 – 6.5	Av x Lid: 4.1E-08
	Lidia	69	4.64 [b]	1.23	2.11	7.46		Lid x Mor: 8.1E-11
	Morucha	102	6.00 [c]	1.27	3.49	10.37		Mor x Av: 0.005
Mg *mg/dL*	Avileña	10	2.60 [a]	0.25	2.29	2.99	1.8 – 2.3	Av x Lid: 0.002
	Lidia	69	2.26 [b c]	0.31	1.45	2.89		Lid x Mor: 0.839
	Morucha	102	2.28 [a c]	0.76	1.20	6.97		Mor x Av: 0.189
Cu *ug/dL*	Avileña	10	798.56 [a]	125.10	568.40	1054	157	Av x Lid: 0.008
	Lidia	67	570.49 [b c]	256.32	44.43	1009		Lid x Mor: 0.091
	Morucha	102	656.18 [a c]	359.92	55.73	1208		Mor x Av: 0.213
Fe *ug/dL*	Avileña	10	165.64	27.95	135.79	233.89	100 - 290	Av x Lid: 0.121
	Lidia	69	149.34	30.67	73.94	232.11		Lid x Mor: 0.549
	Morucha	102	158.96	129.88	67.75	1035		Mor x Av: 0.572
Zn *ug/dL*	Avileña	10	962.78 [a]	90.39	837.60	1140	232	Av x Lid: 5.3E-15
	Lidia	60	142.66 [b]	252.72	13.83	967.74		Lid x Mor: 4.6E-07
	Morucha	102	466.90 [c]	433.45	5.09	1333		Mor x Av: 5.2E-04
Se *ug/dL*	Avileña	10	34.78 [a]	13.63	20.00	61.7	-	Av x Lid: 2.9E-05
	Lidia	64	53.99 [b]	12.29	23.84	79.37		Lid x Mor: 0.005
	Morucha	73	46.89 [c]	16.01	15.80	81.54		Mor x Av: 0.027

The parameters studied are: total proteins; albumins; glucose; cholesterol; creatinine; Urea/BUN; AST (aspartate aminotransferase); ALT (alanine aminotransferase); GGT (gamma-glutamyl transferase); ALP (alkaline phosphatase); CK (creatine kinase); Calcium; Phosphorus; Magnesium; Copper; Iron; Zinc; Selenium. Different letters in each parameter indicate significant media differences ($p < 0.05$).
R. C V indicates the Reference Cattle Values according to Radostits et al., 2006.

The biochemical results according to the sex of the animals are presented in table 18. This table compares males and females from the Lidia breed.

Table 18: Descriptive statistics of Lidia males and females of some biochemical parameters.

Parameter	Lidia	n	Mean	SD	Minimum	Maximum	R. C V	p-value
Total Proteins *g/L*	Males	30	66.37 [a]	9.25	53.16	81.10	57 – 81	0,0002
	Females	67	60.52 [b]	5.46	48.52	74.91		
Albumins *g/L*	Males	30	36.49 [a]	4.85	22.75	44.12	21 – 36	0,0491
	Females	69	38.34 [b]	3.89	26.33	44.92		
Glucose *mg/dL*	Males	30	142.86 [a]	33.89	82.25	199.92	45 – 75	5,11E-06
	Females	69	111.65 [b]	26.99	77.65	214.45		
Cholesterol *mg/dL*	Males	15	76.53 [a]	36.16	29.61	154.72	65 – 220	2,44E-06
	Females	14	157.70 [b]	34.66	108.90	209.59		
Creatinine *mg/dL*	Males	30	1.87	0.34	1.23	2.73	1 – 2	0,2717
	Females	69	1.80	0.29	1.29	2.64		
Urea/BUN *mg/dL*	Males	30	22.33	7.23	12.71	43.02	6 – 28	0,1038
	Females	68	19.31	8.74	7.90	49.62		
AST *U/L*	Males	15	290.42 [a]	123.40	139.53	597.47	78 – 132	0,0002
	Females	14	140.05 [b]	32.96	75.99	205.79		
ALT *U/L*	Males	30	40.01	7.95	24.40	66.49	11 – 40	0,8615
	Females	69	40.33	8.40	22.02	68.62		
GGT *U/L*	Males	30	33.72	20.96	3.70	108.39	6.1 –17.4	0,5376
	Females	69	38.53	39.83	0.29	308.10		
ALP *U/L*	Males	30	136.81	46.30	51.94	227.82	0 - 500	0,3465
	Females	68	116.14	114.37	28.94	710.51		
CK *U/L*	Males	7	462.33	178.76	297.05	859.57	35 - 280	0,9400
	Females	14	477.18	473.64	130.29	1651		
Ca *mg/dL*	Males	30	9.08	0.74	7.25	10.02	9.7 –12.4	0,0832
	Females	69	9.31	0.53	8.17	10.63		
P *mg/dL*	Males	30	4.93	1.19	2.56	7.75	5.6 – 6.5	0,2785
	Females	69	4.64	1.23	2.11	7.46		
Mg *mg/dL*	Males	30	2.11	0.27	1.67	2.93	1.8 – 2.3	0,0648
	Females	69	2.26	0.31	1.45	2.89		
Cu *ug/dL*	Males	30	595.93	333.61	60.29	1199	157	0,6905
	Females	67	570.49	256.32	44.43	1009		
Fe *ug/dL*	Males	30	167.67	69.05	69.77	330.37	100 - 290	0,0734
	Females	69	149.34	30.67	73.94	232.11		
Zn *ug/dL*	Males	29	293.41 [a]	402.99	8.91	1141	232	0,0362
	Females	68	142.66 [b]	252.72	13.83	967.74		
Se *ug/dL*	Males	25	92.36 [a]	53.84	43.82	226.98	-	8,83E-07
	Females	64	53.99 [b]	12.29	23.84	79.37		

The parameters studied are: total proteins; albumins; glucose; cholesterol; creatinine; Urea/BUN; AST (aspartate aminotransferase); ALT (alanine aminotransferase); GGT (gamma-glutamyl transferase); ALP (alkaline phosphatase); CK (creatine kinase); Calcium; Phosphorus; Magnesium; Copper; Iron; Zinc; Selenium. Different letters in each parameter indicate significant media differences ($p < 0.05$).
R. C V indicates the Reference Cattle Values according to Radostits et al., 2006.

The last three tables show the biochemical results of the animals according to their age. Lidia male's results are analyzed in the table 19, Lidia females in the 20 and Morucha females in table 21.

Table 19: Descriptive statistics of yearling and adult Lidia males of some biochemical parameters.

Parameter	Lidia Males	n	Mean	SD	Minimum	Maximum	R. C V	p-value
Total Proteins *g/L*	Yearlings	11	56.62 [a]	2.48	53.16	60.75	57 – 81	1,06E-07
	Adults	19	72.01 [b]	6.70	60.03	81.10		
Albumins *g/L*	Yearlings	11	39.86 [a]	2.17	37.28	44.12	21 – 36	0,0027
	Adults	19	34.54 [b]	4.91	22.75	42.74		
Glucose *mg/dL*	Yearlings	11	133.11 [a]	30.43	100.57	184.64	45 – 75	2,75E-26
	Adults	19	148.51 [b]	34.51	82.25	199.92		
Cholesterol *mg/dL*	Yearlings	-	-	-	-	-	65 – 220	1,63E-06
	Adults	19	76.53	36.16	29.61	154.72		
Creatinine *mg/dL*	Yearlings	11	1.69 [a]	0.16	1.23	1.87	1 – 2	0,0035
	Adults	19	2.00 [b]	0.34	1.45	2.73		
Urea/BUN *mg/dL*	Yearlings	11	18.99	4.54	12.88	28.10	6 – 28	0,0570
	Adults	19	24.26	7.78	12.71	43.02		
AST *U/L*	Yearlings	-	-	-	-	-	78 – 132	-
	Adults	15	290.42	123.40	139.53	579.47		
ALT *U/L*	Yearlings	11	39.41	4.51	31.35	45.60	11 – 40	0,7643
	Adults	19	40.35	9.37	24.40	66.49		
GGT *U/L*	Yearlings	11	19.55 [a]	9.71	3.70	42.94	6.1 –17.4	0,0036
	Adults	19	41.92 [b]	21.34	6.09	108.39		
ALP *U/L*	Yearlings	11	165.88 [a]	29.70	119.06	199.45	0 - 500	0,0075
	Adults	19	119.98 [b]	45.85	51.94	210.37		
CK *U/L*	Yearlings	-	-	-	-	-	35 - 280	-
	Adults	7	462.33	178.76	297.05	859.57		
Ca *mg/dL*	Yearlings	11	9.62 [a]	0.26	9.08	10.02	9.7 –12.4	0,0013
	Adults	19	8.77 [b]	0.75	7.25	9.93		
P *mg/dL*	Yearlings	11	5.60 [a]	0.50	4.89	6.48	5.6 – 6.5	0,0185
	Adults	19	4.54 [b]	1.30	2.56	7.75		
Mg *mg/dL*	Yearlings	11	2.14	0.30	1.77	2.93	1.8 – 2.3	0,9073
	Adults	19	2.13	0.26	1.67	2.50		
Cu *ug/dL*	Yearlings	11	672.67	71.78	589.10	810.30	157	0,3523
	Adults	19	550.87	409.03	60.24	1199		
Fe *ug/dL*	Yearlings	11	119.80 [a]	18.27	95.01	149.07	100 - 290	0,0027
	Adults	19	195.38 [b]	72.40	69.77	330.37		
Zn *ug/dL*	Yearlings	11	21.77 [a]	4.68	15.90	30.83	232	0,0033
	Adults	18	459.42 [b]	434.72	8.91	1140		
Se *ug/dL*	Yearlings	11	58.10 [a]	9.06	43.82	79.05	-	0,0033
	Adults	14	119.28 [b]	58.87	49.50	226.98		

The parameters studied are: total proteins; albumins; glucose; cholesterol; creatinine; Urea/BUN; AST (aspartate aminotransferase); ALT (alanine aminotransferase); GGT (gamma-glutamyl transferase); ALP (alkaline phosphatase); CK (creatine kinase); Calcium; Phosphorus; Magnesium; Copper; Iron; Zinc; Selenium. Different letters in each parameter indicate significant media differences ($p < 0.05$).
R. C V indicates the Reference Cattle Values according to Radostits et al., 2006.

Table 20: Descriptive statistics of Lidia 2 years, 3 years and adult females of some biochemical parameters.

Parameter	Lidia Females	n	Mean	SD	Minimum	Maximum	R. C . V	p-value
Total Proteins *g/L*	2 years	10	53.04 [a]	1.78	48.52	55.56	57 – 81	2 x 3: 3.6E-09
	3 years	10	61.50 [b]	1.60	59.30	64.75		3 x A: 0.812
	Adults	47	61.91 [b]	5.21	53.84	74.91		A x 2: 2.8E-06
Albumins *g/L*	2 years	10	35.40 [a]	3.47	28.81	39.66	21 - 36	2 x 3: 0.018
	3 years	10	39.38 [b]	2.98	35.38	43.37		3 x A: 0.63
	Adults	49	38.73 [b]	3.86	26.33	44.92		A x 2: 0.016
Glucose *mg/dL*	2 years	10	141.60 [a]	41.83	101.02	214.45	45 - 76	2 x 3: 0.133
	3 years	10	116.63 [a c]	22.77	81.75	155.32		3 x A: 0.075
	Adults	49	104.52 [b c]	18.00	77.65	176.34		A x 2: 4.5E-05
Cholesterol *mg/dL*	2 years	-	-	-	-	-	65 - 220	2 x 3: -
	3 years	-	-	-	-	-		3 x A: -
	Adults	14	157.70	34.66	108.90	209.59		A x 2: -
Creatinine *mg/dL*	2 years	10	1.59 [a]	0.11	1.43	1.81	1 – 2	2 x 3: 0.656
	3 years	10	1.57 [a]	0.12	1.29	1.79		3 x A: 0.002
	Adults	49	1.88 [b]	0.30	1.37	2.64		A x 2: 0.004
Urea/BUN *mg/dL*	2 years	10	19.61	7.97	12.59	41.69	6 - 28	2 x 3: 0.46
	3 years	10	22.11	5.96	9.98	29.99		3 x A: 0.27
	Adults	48	40.59	8.90	7.90	49.62		A x 2: 0.77
AST *U/L*	2 years	-	-	-	-	-	78 - 132	2 x 3: -
	3 years	-	-	-	-	-		3 x A: -
	Adults	14	140.05	32.96	75.99	205.79		A x 2: -
ALT *U/L*	2 years	10	40.74	7.69	29.01	51.85	11 - 40	2 x 3: 0.528
	3 years	10	38.65	5.96	30.36	47.95		3 x A: 0.52
	Adults	49	40.59	8.90	22.02	68.62		A x 2: 0.96
GGT *U/L*	2 years	10	20.61 [a]	6.51	7.87	30.29	6.1 – 17.4	2 x 3: 0.004
	3 years	10	37.27 [b c]	13.81	16.42	59.61		3 x A: 0.73
	Adults	49	42.44 [a c]	45.88	0.29	308.10		A x 2: 0.145
ALP *U/L*	2 years	10	151.38 [a]	85.92	76.71	356.24	0 – 500	2 x 3: 0.336
	3 years	9	220.77 [a]	189.09	62.16	710.51		3 x A: 0.002
	Adults	49	89.73 [b]	84.36	28.94	620.04		A x 2: 0.044
CK *U/L*	2 years	-	-	-	-	-	35 - 280	2 x 3: -
	3 years	-	-	-	-	-		3 x A: -
	Adults	14	477.18	473.64	130.29	1651		A x 2: -
Ca *mg/dL*	2 years	10	9.27	0.37	8.65	9.78	9.7 – 12.4	2 x 3: 0.27
	3 years	10	9.48	0.43	8.97	10.36		3 x A: 0.31
	Adults	49	9.29	0.57	8.17	10.63		A x 2: 0.91
P *mg/dL*	2 years	10	6.41 [a]	0.65	5.32	7.64	5.6 – 6.5	2 x 3: 9E-04
	3 years	10	4.72 [b]	1.10	3.17	6.71		3 x A: 0.21
	Adults	49	4.26 [b]	1.01	2.11	6.62		A x 2: 4.1E-08
Mg *mg/dL*	2 years	10	1.92 [a]	0.19	1.60	2.28	1.8 – 2.3	2 x 3: 0.219
	3 years	10	2.07 [a]	0.31	1.45	2.66		3 x A: 0.002
	Adults	49	2.37 [b]	0.26	1.74	2.89		A x 2: 2.4E-06
Cu *ug/dL*	2 years	10	685.23 [a]	63.39	606.29	806.96	157	2 x 3: 0.079
	3 years	10	769.62 [a]	120.31	588.71	1009		3 x A: 0.004
	Adults	47	503.71 [b]	272.07	44.43	869.25		A x 2: 0.044
Fe *ug/dL*	2 years	10	127.36 [a]	20.93	76.65	152.35	100 - 290	2 x 3: 0.13
	3 years	10	146.81 [a c]	30.27	101.95	187.39		3 x A: 0.485
	Adults	49	154.35 [b c]	30.38	73.94	232.11		A x 2: 0.011
Zn *ug/dL*	2 years	9	40.13	9.59	28.21	61.82	232	2 x 3: 0.288
	3 years	10	44.75	7.73	34.81	61.68		3 x A: 0.135
	Adults	41	189.05	294.34	13.83	967.74		A x 2: 0.144
Se *ug/dL*	2 years	10	52.80 [a]	6.38	39.64	61.70	-	2 x 3: 2.6E-04
	3 years	10	65.59 [b]	5.56	58.53	76.33		3 x A: 0.002
	Adults	44	51.56 [a]	12.91	23.84	79.37		A x 2: 0.783

The parameters studied are: total proteins; albumins; glucose; cholesterol; creatinine; Urea/BUN; AST (aspartate aminotransferase); ALT (alanine aminotransferase); GGT (gamma-glutamyl transferase); ALP (alkaline phosphatase); CK (creatine kinase); Calcium; Phosphorus; Magnesium; Copper; Iron; Zinc; Selenium. Different letters in each parameter indicate significant media differences ($p < 0.05$).
R. C V indicates the Reference Cattle Values according to Radostits et al., 2006.

Table 21 : Descriptive statistics of Morucha 2/3 years and adult females of some biochemical parameters.

Parameter	Morucha Females	n	Mean	SD	Minimum	Maximum	R. C V	p-value
Total Proteins *g/L*	2/3 years	22	75.76	7.39	65.90	92.80	57 – 81	0,4136
	Adults	80	74.64	5.12	61.69	90.20		
Albumins *g/L*	2/3 years	22	40.41	3.14	35.06	47.90	21 – 36	0,5874
	Adults	79	39.61	6.70	17.06	60.57		
Glucose *mg/dL*	2/3 years	22	104.16 [a]	28.68	45.10	153.90	45 – 75	0,0158
	Adults	78	83.74 [b]	35.84	16.95	219.17		
Cholesterol *mg/dL*	2/3 years	22	178.82	28.88	134.80	225.30	65 – 220	0,9267
	Adults	47	179.75	42.92	119.88	302.10		
Creatinine *mg/dL*	2/3 years	22	1.89 [a]	0.36	1.30	2.70	1 – 2	0,0249
	Adults	80	2.17 [b]	0.56	1.18	3.59		
Urea/BUN *mg/dL*	2/3 years	22	15.75	5.57	5.80	23.50	6 – 28	0,6677
	Adults	80	15.02	7.36	3.80	37.45		
AST *U/L*	2/3 years	22	108.13	16.11	84.73	147.79	78 – 132	0,9070
	Adults	75	108.90	29.17	64.61	215.39		
ALT *U/L*	2/3 years	22	44.00	7.49	28.61	58.41	11 – 40	0,9850
	Adults	76	43.83	41.79	14.59	365.37		
GGT *U/L*	2/3 years	22	6.04 [a]	6.74	0.10	26.90	6.1 –17.4	0,0007
	Adults	63	11.93 [b]	6.77	1.94	53.12		
ALP *U/L*	2/3 years	22	77.33	34.51	42.23	164.56	0 - 500	0,9332
	Adults	76	75.48	32.13	17.19	594.65		
CK *U/L*	2/3 years	-	-	-	-	-	35 - 280	-
	Adults		432.62	406.45	82.05	2365		
Ca *mg/dL*	2/3 years	22	9.99	0.85	9.20	13.20	9.7 –12.4	0,9312
	Adults	80	9.96	1.10	8.12	13.16		
P *mg/dL*	2/3 years	22	6.39	1.13	4.80	9.30	5.6 – 6.5	0,1050
	Adults	80	5.89	1.30	3.49	10.37		
Mg *mg/dL*	2/3 years	22	2.21	0.29	1.60	2.70	1.8 – 2.3	0,6292
	Adults	80	2.30	0.85	1.20	6.97		
Cu *ug/dL*	2/3 years	22	872.56 [a]	120.26	659.46	1063	157	0,0010
	Adults	80	596.67 [b]	375.89	55.73	1208		
Fe *ug/dL*	2/3 years	22	132.23	30.03	77.70	184.00	100 - 290	0,2802
	Adults	80	166.31	145.89	67.75	1035		
Zn *ug/dL*	2/3 years	22	888.57 [a]	158.50	642.39	1151	232	4,32E-08
	Adults	80	350.94 [b]	415.65	5.09	1333		
Se *ug/dL*	2/3 years	22	55.22 [a]	8.59	41.77	73.43	-	0,0030
	Adults	51	43.29 [b]	17.31	15.80	81.54		

The parameters studied are: total proteins; albumins; glucose; cholesterol; creatinine; Urea/BUN; AST (aspartate aminotransferase); ALT (alanine aminotransferase); GGT (gamma-glutamyl transferase); ALP (alkaline phosphatase); CK (creatine kinase); Calcium; Phosphorus; Magnesium; Copper; Iron; Zinc; Selenium. Different letters in each parameter indicate significant media differences ($p < 0.05$).
R. C V indicates the Reference Cattle Values according to Radostits et al., 2006.

5. Discussion

Before starting the analysis of the results, it is essential to refer to a very important aspect: the samples collected for this study were performed over several years and at different periods of the year, with the animals in different stages of production and body condition. This aspect, together with the fact that the breeds studied are rather heterogenous breeds and with a large intra-racial variety could cause variations in some results obtained.

5.1 Breed effect on hematological values

Both WBC (white blood cells) and RBC (red blood cells) are very important in animal health. While WBC is part of the immune system and helps the animal fight diseases and infections, RBC is essential to the transport of oxygen and the removal of carbon dioxide from cells. In this study, significant differences were observed only when comparing females of different breeds. In both cases, the levels in the Morucha breed were higher than the Lidia breed (19% in relation to the WBC and 6% to the RBC). Hernández in 1992, studying the Morenas Gallega's breeds, obtained values between 7.32 and 8.76 ($*10^6$) for WBC and between 7.41 and 9.00 ($*10^6$) for the RBC. Comparing to our results, we observed that only the values relative to the WBC content in the Avileña breed are within the intervals obtained by Hernández. Higher WBC values might be associated with a recent infection or disease or a result from a side effect of a medicine while lower values could indicate a lower efficiency in immune system action. Higher RBC values in certain breeds might be associated with a more accelerated metabolism or with animals living at higher altitudes. In both cases there is a need to perform gas exchange at a higher rate.

Hemoglobin is a protein present in red blood cells that allows oxygen transport through the circulatory system while hematocrit values refer to the percentage of volume occupied by RBC in the total blood volume. Hemoglobin results showed significant differences only when analyzed all animals belonging to the study. In this case, the Lidia breed presented higher levels of hemoglobin compared to the Morucha (approximately 6% higher). On the other hand, the hematocrit results showed that the Morucha breed had between 7-10% higher values than those of Lidia breed. Hernández (1992) obtained values between 13.08 and 15.13 (g/L) for hemoglobin and between 42,75 and 51,28 (%) for hematocrit. In both cases we obtained values lower than those of this author (except for the Hb value of Lidia's animals that was within the range described by Hernández). Given their function, lower hemoglobin levels might be associated with a less accelerated metabolism since oxygen transport is done at lower intensities. These values may also be caused by situations of anemia or hemolytic diseases. Lower hematocrit values represent lower erythrocyte volumes which could mean lower efficiency in gas exchange.

MCV (Medium corpuscular Volume) measures the average size of the red blood cells while MCH (Medium corpuscular hemoglobin) allow us to know the average amount of hemoglobin found in each red blood cell. When we observed the results of these hematological parameters as a function of the breed, we concluded that MCV values were significantly higher in the Morucha breed compared to the others. On the other hand, it was the Lidia breed that had significantly higher MCH values. In 1982, Prieto et al., working with Retinta animals in Andalusia, obtained a mean MCV value of 48.05 fL, lower than those found in the three studied breeds. For MCH values we did not find values from other studies. Higher MCV values can represent larger red blood cells and, maybe, capable of carrying larger volumes of gases. These values can also be associated with an macrocytic anemia due to lack of vitamin B12 or folic acid or may simply be a normal value for the breed. In relation to MCH, higher values indicate higher amounts of hemoglobin per erythrocyte, which may contribute to the increase in the amount of oxygen that each RBC can carry.

The mean corpuscular hemoglobin concentration (MCHC) refers to the average hemoglobin in 1 dL of erythrocytes. It is a more accurate and reliable measure than MCH. The results associated with this parameter showed differences between the three breeds studied. The Lidia presented the highest values, followed by the Avileña breed, being the Morucha the one who presented the lowest values. Breeds with higher MCHC levels have higher hemoglobin concentrations in their erythrocytes, being representative of rustic breeds, highly adapted to the environment and with a great capacity to move and walk-through difficult terrain. This can contribute to increased efficiency in the transport of oxygen through cells. As with the MCH, we found no results from other authors with whom we could compare ours.

Regarding blood platelets, they are very important in the blood clotting process. In relation to the differences between the breeds studied, we could only observe that the Morucha presented approximately 40 % higher values than the Lidia. A higher level of blood platelets indicates a greater ability of the blood to clot. There are no studies with which we can compare these values, but it would be interesting in future studies to try to understand the reason for such a marked difference between the Morucha and Lidia breeds and also to understand the impacts that this can have. Our experience does not show that the measurements of platelets is not always completely correct, which could be due to microclots that are produced when sampling the animals.

5.2 Sex effect on hematological values

Regarding WBC (white blood cells) there were clearly significant differences since, Lidia males had approximately 30% higher values than females. We think that these differences may be associated with the greater stress that males have chronically and also the greater dificculty in handling. These results were different from those obtained by Birgel et al. (1991), since these authors did not observe significant differences between males and females of the Jersey breed.

When it comes to red blood cells, we obtained some surprising results. Indeed, when comparing the RBC concentrations between yearling males and females we observe that there are no significant differences. However, when we evaluate these values in older animals, it is clear that males have significantly higher levels of erythrocytes than females, possible due to stress and physical exercise caused by reproductive breeding and fights between them. These results are in line with those obtained by Birgel et al. (1991), since these authors also observed higher levels of erythrocytes in males and can possibly be due to a higher level of physical activity in males when compared to females.

When we analyzed hemoglobin values, we observed similar results to those of the red blood cells, and they should evolve in parallel. In yearling animals, no significant differences were observed. However, from that age, males have significantly higher levels. Such results can be explained as hemoglobin is a protein that is present in RBC. Since differences in RBC levels between males and females were observed only from the year of age, it would be expected that the same would happen with hemoglobin. On the one hand, our results are in accordance with those obtained by Doornenbal (1977) and by Doornenbal et al. (1998) in beef cattle breeds. On the other hand, they contrast with those obtained by Birgel et al. in 1991 once they demonstrated that females had superior levels of hemoglobin.

Since hematocrit corresponds to the percentage of volume occupied by RBCs in the total volume of blood it would be expected that the results would be similar. Similarly to erythrocytes, there were no significant differences between males and females in the percentage of hematocrit in yearling animals although, from this age, males assumed significantly higher values. These results are in line with those obtained by Birgel et al. (1991) despite contradicting those obtained by Dornenbal (1977) and Doornenbal et al. (1988). Such discrepancies are likely due to the nature of these animals, where males have been selected for higher physical activity and competitiveness.

The MCV values were not significantly affected by the sexual factor, contrary to what was observed by Doornenbal in 1977, whose results showed significantly higher values in females. The results for MCH and MCHC were similar. In both cases, there were no significant variations in yearlings. In older animals, males obtained significantly higher levels than females. Unlike hemoglobin and hematocrit, the results of MCH and MCHC cannot be justified by the increase of RBC in males older than one year, since these values relate to the average amount and concentration of hemoglobin in each red blood cell. The results of these parameters indicate a higher amount and concentration of hemoglobin present in erythrocytes that may increase the oxygen transport efficiency.

Considering the blood platelets values, and contrary to what was found in the red blood series, we observed significant differences in yearling animals since females have significantly

higher values. When comparing older animals we notice that these differences dissipate. This may indicate that platelet production stabilizes earlier in females than in males.

5.3.Age effect on hematological values

Regarding the WBC (white blood cells), we observed an increase as the age progresses in the Lidia male case. These results agrees with the results obtained by Hernández, 1992 and by Pereira et al. (1987), who observed an increase in this value with age. On the other hand, Birgel et al. (2001) observed an increase in the number of WBC until the age of two, although from this age this value decreased.

Hernández (1992) and Pereira et al. (1987), showed a decreasing trend in the amount of red blood cells as the animals age. The results obtained in Lidia females follow the same line as those of the above-mentioned authors since adults had significantly lower erythrocyte levels than the younger animals. Regarding the Morucha females' results values oscillated irregularly which did not allow to determine a clear trend.

The values related to the evolution of hemoglobin with age did not allow us to establish a typical pattern, due to irregular variations (although there has been a significant difference in Lidia males, since these values were higher in adults). The same was concluded in the studies of Pereira et al. (1987) and Hernández (1992), although Prieto et al. (1982) found a slight decreasing trend in his study with Retinta animals.

Hernández (1992) and Prieto et al. (1982) observed a negative correlation between hematocrit value and age. In the present case, we obtained results more similar to those of Pereira et al. (1987), since we did not observe a definite trend (although in Morucha females, adults presented significantly lower values than younger animals).

Like Pereira et al. (1987) and Prieto et al. (1982) the results of Lidia females for the medium corpuscular value (MCV) were higher in older animals, contrasting to the results of the Morucha females where the highest values were recorded in younger animals. This means that in the case of Lidia females, erythrocytes tend to be larger in adults, while in the case of Morucha females, are the youngest animals that have the largest RBC.

The MCH, MCHC and platelets values did not allow any conclusion to be taken once there were not any clear trend.

5.4 Breed effect on biochemical values

The measurement of the concentration of total proteins concerns the total amount of protein in the blood, namely two major groups: albumins and globulins. Albumins are important not only for the transport of multiple substances throughout the body and to maintain oncotic

pressure, but also to contribute to the growth and healing of tissues (Kaneko et al, 2008; Dhinna & Palasinamy, 2010; Constable et al, 2017). Regarding the results, the Lidia breed had the lowest values, followed by Morucha and the Avileña breed, which obtained the highest levels. In 1992 Hernández obtained concentrations between 67.7 and 75.23 g/L for TP and 25.34 and 27.31 g/L for albumin. Except for the total protein values in the Morucha breed, all values are outside the intervals described by Hernández. Although protein higher (or lower) values can be indicators of some kind of diseases or simply dehydration, we believe that the differences between breeds on the results analyzed from our study or Hernández's results are related to the variability of the parameters considering, for example, breed or diet (Lérias et al., 2015).

In glucose, the levels present in Lidia's animals were higher than those of Morucha breed (about 21-27% higher). When we compare our results with those of other authors such as Prieto et al (1982) (glucose values between 54.4 and 64.6 mg/dL for Retinta breed) and Hernandez (1992) with values between 45.09 and 49.35 mg/dL, we observe that our values are much higher. Higher glucose values may be related to higher energy values in diets, physical activity or the timing of sampling but especially with the stress caused by sampling, particularly in more indocile breeds, such as Morucha or Lidia. Nevertheless, it would be interesting in future studies to understand if the differences between the glucose values obtained in our study and those obtained by other authors can be fully justified by the energy level present in the diets of animals.

In the case of cholesterol, there were only differences when compared all animals, and the Morucha breed had values 36% higher than Lidia. Hernández in 1992 obtained values between 109.2 and 132.72 mg/dL cholesterol for breeds belonging to the *Morenas del Noroeste.* Only the values in Lidia animals (males and females) were within the interval obtained by Hernández, all other values were higher. Higher cholesterol levels may be related to higher intake of fat and consequent absorption (Aboelmaty et al., 2008) or to a negative energy balance that requires the mobilization of fat as an energy source (Kaneko et al, 2008; Constable et al, 2017).

Urea and creatinine are two very important parameters to evaluate the performance of the animals' renal function. Creatinine is a nitrogenous substance formed from the muscle metabolism of creatine and phosphocreatine, and is subsequently eliminated by urine (Gregory et al., 2004). Urea is an important source of nitrogen, through its direct intake in the diet or its reentrance in the rumen across the ruminal epithelium (Huntington & Archiberque, 1999). Regarding creatinine, the Morucha breed presented the highest values and Avileña the lowest while in urea it was the Avileña who obtain the highest values and the Morucha the lowest. When comparing our results to those of Gregory et al. (2004), (creatine values: 1.33- to 1.43 mg/dL and urea values:19.27-21.12 pg in Jersey cattle, and those of Hernández (1992) (creatine levels: 1.23-1.43 mg/dL and urea levels: 18.02-25.02 mg/dL), we observed that the creatinine levels obtained in our study were higher. The levels of urea of the Lidia breed were the only ones similar to those of Hernández and Gregory et al. (2004) since the levels of the Morucha breed were lower and the Avileña's ones were higher. One of the reasons for the variation in the values of these parameters may be the fact that both are metabolites with high variation according to breed and on a more or

less protein diet (Liu & McMeniman, 2006; Kaneko et al, 2008; Lérias et al., 2015; Constable et al, 2017).

In relation to enzymes, aspartate aminotransferase (AST), that plays an important role in metabolization of amino acids (Washington & Hoosier, 2012) showed significantly higher values in the Lidia breed, followed by Morucha and finally by Avileña. Concerning gamma-glutamyl transferase (GGT), is a membrane-bound enzyme which function is based in secretion and resorption of amino acids (Stojevic et al., 2005). In our results, the Avileña and Lidia showed higher values with statistical significance compared to the Morucha. According to Doornenbal et al. (1988), alkaline phosphatase an enzyme which catalyzes the liberation of phosphorus from phosphate esters. The results for ALP allowed us to verify that the Lidia animals had higher blood concentrations than the Morucha's (about 35-38% higher). Overall, our results for these three enzymes were shown to be much higher than those obtained by Hernández in 1992 (AST levels: 47.54-53.54 U/L; GGT levels: 13.02-16.15 U/L; ALP levels: 37.14-48.84 U/L). The only exception concerns the levels of GGT in Morucha animals that shown to be inferior not only to the other breeds studied but also to the results obtained by Hernández.

According to Washington and Hoosier (2012), calcium, phosphorus and magnesium are very important to the organism. Calcium is the most prevalent mineral in the body and is involved in bone tissue metabolism and conduction of electrical impulses. Phosphorus is essential for the formation of bones, teeth and proper functioning of the Krebbs cycle. Magnesium is involved in enzyme reactions, conduction of electrical stimuli, and bone formation (Suttle, 2011). Calcium levels were significantly higher in the Avileña and Morucha breeds compared to Lidia. The results for inorganic phosphorus show that the Avileña breed had the highest values and Lidia the lowest. Regard the levels of magnesium we could verify that the Avileña breed presented statistically higher values than the Lidia (approximately 15%). It is concluded that the Lidia breed has lower levels of these minerals. The main reason for the difference in the values of these macrominerals is related to diet. It is difficult for grazing or even browsing, to be able to meet the needs of Ca, P and Mg. Therefore, farmers must supplement the diet with concentrates and forages that provide these minerals, and even add vitamin-mineral correctors. When comparing our results with those obtained by Hernández (1992), we observed that: with the exception of the Avileña breed, whose values were higher, the calcium results are within the range described by the author for Spanish autochthonous breeds belonging to the Morenas del Noroeste group. That is between 8.69 and 9.51 mg/dL; the phosphorus levels of the Lidia animals were similar to those of Hernández (1992) (4.45 – 5.09 mg/dL), while those of the Avileña and Morucha breeds were higher. Concerning the magnesium levels, our results were lower than the interval obtained by that author (2.36 - 2.78 mg/dL), except for the Avileña animals that presented values within such range.

In trace elements, there were differences in three minerals: copper, zinc and selenium. Copper is an essential component for the activity of a large number of enzymes (Gilbert, 1952; Kaneko et al, 2008; Suttle, 2010). Zinc has long been established as an important element, being involved in protein synthesis, carbohydrate metabolism and many others biochemical reactions

(Miller, 1970; Suttle, 2010). Selenium plays an equally important role in several cell metabolism, such as cell and mitochondrial membranes protection (by promoting glutathione peroxidase synthesis) and preventing thyroid damages (Kaneko et al, 2008; Suttle, 2010; Constable et al, 2017). Our results showed that Avileña breed presented statistically higher copper values than those of the Lidia breed (30% higher). The results of zinc and selenium were the opposite. While zinc values were maximum in the Avileña breed and minimum in the Lidia breed, selenium values were higher in the Lidia and lower in the Avileña. There is not much data with which we can compare our values. Despite this, the results related to copper showed to be quite superior to those obtained by Hernández, 1992 (81-112 ug/dL). Regarding the zinc results, the Lidia breed showed lower values than those of Hernández (220-243 ug/dL) while the Avileña and Morucha breeds obtained much higher values. Selenium deficiency is common in the Iberian Peninsula, for this reason the diet is supplemented with this mineral, and it is even applied parenterally to newborn animals, their mothers and reproducers, coinciding with other manipulations such as deworming or sanitary samplings.

5.5 Sex effect on biochemical values

Within the biochemical variables, significant differences were observed in total proteins (TP), albumins (Alb), glucose (Glu) and cholesterol (Chol). In the case of TP males, they presented significantly higher values (9% higher) while females obtained 5% higher albumin contents. Our results were similar to those obtained by Doornenbal et al. (1988), since these authors also observed higher levels of TP in males and albumins in females. These differences may be related to the fact that males have more protein-rich diets due to the need to form a strong muscle structure that allows them to be in ideal physical conditions for the bull fighting (the *lide* in Potuguese or *lidia* in Spanish).

Glucose values were significantly higher in males (about 22%). This difference may be associated with stress promoted by sample collection. These results are in line with those obtained by Walker et al. (2010) although Doornenbal et al. (1988) recorded slightly higher glucose values in females. In the case of cholesterol, females presented higher values. All these bovine females show a negative energy balance in pregnancy and lactation, which can be more or less intense. When it is not compensated, they are forced to metabolize fat reserves (Kaneko et al, 2008; Constable et al, 2017).

Regarding enzymes, we only observed differences with statistical relevance in AST, with males presenting 52% higher levels than females. This may be because Lidia males require much higher amounts of protein in their diet due to the need to form a strong muscle and skeleton, especially during the year prior to the fight when the Lidia bulls receive an extra supplement called *acabado* . As AST plays an important role in the metabolization of amino acids (Washington and Hoosier, 2012), a higher amount of protein ingested may be associated with a higher content of

this enzyme in the blood. The bulls stress, the frequent fights, etc. can cause muscle dysfunction, which can also increase the values of certain enzymes such as AST.

Sex does not seem to influence blood levels of any macrominerals studied, being more attributed to the effect of diet and supplementation. In the trace elements there were significant differences in zinc and selenium contents, and, in both cases, higher values were found in males (about 51% higher zinc and 42% higher selenium values). We believe that further studies could be carried out on this subject, as there are no major studies on the impact of sex on the concentrations of these trace elements

5.6 Effect of age on biochemical values

Total protein (TP) values increased significantly with age (except for the females of the Morucha breed where age did not significantly affect TP levels). These results are similar to those obtained by Hernández (1992), Peterson & Walden (1981) and Doornenbal et al. (1998). One of the reasons that can explain these values may be the fact that older animals have a more developed muscular system than younger ones, which implies greater protein requirements in their diet that culminates in a higher blood protein content. In addiction it is possible that the steers receive worse feeding due to the competition with the mothers and with other sterers of similar age.

As far as albumins are concerned, it was not possible to draw conclusions as variable results were obtained. When analyzing the Lidia male's values, we observed higher levels in younger animals, contrasting with those obtained in Lidia females where older animals showed significantly higher concentrations. In 1992, Hernández faced the same scenario since in their study, the Vianese and Limiana breeds presented higher albumin levels in younger animals and the Cachena and Caldelana breeds in older animals. These results allow us to conclude that higher TP levels in adult males could be related to higher globulin levels.

Like albumin results, glucose values have not allowed us to establish a behavioral trend of this blood parameter with age. On the one hand, in Lidia males, glucose showed significantly higher values in older animals. On the other hand, females of the Lidia and Morucha breeds, presented higher levels in younger animals. Analyzing the studies already carried out on this subject, we can observe that Peterson & Walden (1981) obtained similar results to those of the Lidia males, while Hernández (1992) and Barnes et al. (1985) concluded that glucose levels tend to decline with age. Again these results are a likely consequence of the very diverse environments and timing in which these results were obtained.

Creatinine levels were significantly higher in older animals, agreeing with the results obtained by Gregory et al. (2004) but contrary to those of Hernández (1992). In our view these results make sense since, creatinine is a substance produced by the muscles and adult animals

have a more developed muscular skeleton compared to younger animals. In addition to that, older animals have more fights between them which can cause tissue injuries and contribute to the increase of creatinine levels.

As regards enzymes, age affected the contents of only two: gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP). In the cases of Lidia males and Morucha females, GGT concentrations were higher in older animals, possible due to increased liver impairment. The same was observed by Hernández (1992), where older animals had higher GGT concentrations. According to Washington & Hoosier (2012), bone growth in young animals produces elevated ALP level. Therefore, it could be expected that younger animals would have higher ALP values than older animals, as they have higher yields of bone tissue. The results showed precisely what was expected, once the concentrations of this enzyme were significantly higher in younger animals.

In relation to macrominerals, both calcium and phosphorus showed decreasing tendencies with age. These results were expected since these minerals are absorbed in the intestine or mobilized from the bones and, as age progresses, the absorption capacity of the organs as well as bone mobilization decreases, leading to lower blood concentrations (Hernández, 1992; Suttle, 2010). In the case of magnesium, and although Lidia males had slightly higher values in younger animals, Lidia and Morucha females had higher levels in older animals. Hernandez (1992), observed the same result in the Cachena and Caldelana breeds. Further studies are needed to understand why this event happened since, like calcium and phosphorus, magnesium is also absorbed at an intestinal level and, therefore, it would be expected that the concentration of this macromineral decrease with age.

Concerning trace elements, copper results allowed to verify that the adult animals presented values significantly lower than the younger animals (except in the comparison between yearling and adult Lidia males where there were no significant differences). In the opposite direction, iron levels have assumed a growth trend with age in Lidia males. As we mentioned in the discussion about the results related to the influence of sex in the trace elements, we did not find results with which we could compare our values. This could be also a subject addressed in future studies.

6. Conclusions and future prospects

In general we could observe that the serological parameters studied are quite variable according to breed, sex and age.

Regarding the breeds, all hematological parameters evaluated (white blood cells, red blood cells, hemoglobin, hematocrit, medium corpuscular volume, medium corpuscular hemoglobin, medium corpuscular hemoglobin concentration and platelets) were statistically different between the breeds. In relation to biochemical parameters, only the creatine kinase enzyme and the Iron were the metabolites that did not present statistical differences between the breeds studied.

The results related to the influence of sex allowed us to conclude that regarding the hematological parameters males obtained superior white blood cells values compared to females in the two cases studied (Lidia: males vs females; Lidia yearlings: males vs females). In the biochemical values, males presented higher values of total proteins, glucose, AST, zinc and selenium while females obtained higher values of albumin and glucose.

Age has also shown to be a source of variation in the concentrations of the measured metabolites. In the case of hematological parameters we were able to verify that the variations in the parameters were different according to the comparisons we made (Lidia males: Yearlings vs Adults; Lidia Females: Yearlings vs 2 years vs Adults; Morucha Females: Yearlings vs 2-year vs 3-years vs Adult). For example, in the case of MCV no differences were observed between Lidia males of different ages. In Lidia females the older animals showed higher values while in the Morucha females were the younger animals that presented higher levels. The same happened when we analyzed the biochemical values with the exception of Urea BUN and ALT enzyme where age did not affect the concentrations in none of the analyzed cases (Lidia males: Yearlings vs Adults; Lidia Females: Yearlings vs 2 years vs Adults; Morucha Females: 2/3-years vs Adult) and creatinine, whose values where higher in adult animals.

It would be interesting in the future to carry out work that could give a sequence to our study. These studies could, on the one hand, investigate the differences in blood parameters between the breeds we studied and other breeds more used in industry, such as Angus, Limousine or Charolais. On the other hand, it would be also interesting to extend this study to other Iberian breeds in both Portugal and Spain as initially planned. Finally, it would be interesting to study the differences between the Spanish Lidia breed, the Portuguese Brava de Lide and the Brava de Lide dos Açores.

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