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Assessment of the production system, transportation, and slaughter in a sample of Barrosã PDO herds

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Área científica: Saúde Pública; Produção e/ou Nutrição Animal

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ABSTRACT

Barrosã is a Portuguese cattle breed, characteristic of the Barroso Region, located in the north of Portugal. It is renowned for its ability to produce high organoleptic quality meat recognized with Protected Designation of Origin (PDO). The objective of this study was to characterise the Barrosã production system and its particularities, as well as the transportation to the slaughterhouse and the slaughter. Furthermore, the aim was to identify the variables responsible for the rise in Barrosã PDO meat pH, which is an indicator of carcass quality, using data recorded since the year 2020. Three distinct questionnaires were created and used to characterize, respectively, a sample of farms, the transportation, and the slaughterhouse work. Nineteen Barrosã farms were visited and evaluated, and two transports from the farms of origin to the slaughterhouse were assessed. The work at the slaughterhouse was accompanied for eleven days, during which time a total of 61 Barrosã calves were slaughtered. A data analysis of the Barrosã slaughter records from May 2020 to May 2024 was conducted to characterise the problematic pH results. Furthermore, the Barrosã breeders' association, Associação dos Criadores de Bovinos da Raça Barrosã (AMIBA), database was analysed in order to gain insight into the actual production system situation. The questionnaire results revealed the diversity of the Barrosã production system, encompassing aspects such as feeding, housing, sanitary measures, breeding methods, and differences in transport conditions, duration and routes. Despite the inability to identify the variables responsible for the elevated meat pH, due to this work's limitations, this study demonstrated that the meat pH at 24 hours post-mortem is an inadequate indicator of carcass quality, as the pH decline is not completed. Instead, other timing for pH measurements should be considered, for example at 48 hours post-mortem.

KEYWORDS: Barrosã cattle; meat quality; slaughter; transportation; production system.

RESUMO

Barrosã é uma raça de bovinos portuguesa, característica da região do Barroso, no norte de Portugal. É conhecida pela sua capacidade de produzir carne de alta qualidade organolética, reconhecida com a distinção Denominação de Origem Protegida (DOP). O objetivo deste estudo é caracterizar o sistema de produção do Barroso e as suas particularidades, bem como caracterizar o transporte para o matadouro e o abate e identificar as variáveis responsáveis pelo aumento do pH da carne Barrosã DOP, um indicador da qualidade da carcaça, a partir dos registos feitos desde 2020. Foram criados três questionários distintos aplicados às explorações, ao transporte e ao trabalho no matadouro. Foram visitadas e avaliadas dezanove explorações de carne Barrosã e avaliados dois transportes desde as explorações de origem até ao matadouro. O trabalho no matadouro foi acompanhado durante onze dias, num total de 61 vitelos Barrosã abatidos. Foi também realizada uma análise dos registos dos abates da raça Barrosã no período de Maio 2020 até Maio 2024, de forma a caracterizar os resultados problemáticos de pH. Para além disto, a base de dados da Associação de Criadores de Bovinos da Raça Barrosã (AMIBA) foi analisada para descrever o sistema produtivo da raça Barrosã atual. Os resultados dos questionários revelaram a diversidade deste sistema produtivo, englobando a alimentação, o alojamento, as medidas sanitárias, os métodos de reprodução usados e as distintas condições, durações e rotas dos transportes para o matadouro. Embora, devido às limitações deste trabalho, não tenha sido possível identificar as variáveis responsáveis pela elevação do pH da carne, este estudo demonstrou que a medição do pH às 24 horas post-mortem é um indicador inadequado da qualidade da carcaça, uma vez que a descida do pH não está completa. Ao invés, deve ser considerado outro momento para a mediação do pH, como por exemplo, às 48 horas post-mortem.

PALAVRAS-CHAVE: Gado Barrosão; qualidade da carne; abate; transporte; sistema produtivo.

CASUISTIC DESCRIPTION:

A preliminary literature review was conducted on the topic of this thesis using the PubMed database to search articles containing the keywords: “bovine”; “meat”; “pH”; “pH decline”; “slaughter”; “slaughterhouse”; “transport”; “transport by road”; “animal welfare”; “stress”; “stressors”; and “beef”. This yielded a total of twenty-eight articles, which were subsequently read, analysed, and summarized.

Subsequently, three distinct questionnaires were devised, pertaining to the Barrosã farms, the slaughterhouse work, and the transportation of animals to the slaughterhouse. These were applied *a posteriori*.

The subsequent step was to accompany and characterise the work at the slaughterhouse, applying the previously prepared questionnaire and measuring the meat pH of the Barrosã DOP carcasses. This resulted in 235 pH measurements (111 on the loin region and 135 on the chuck region) on a total of 61 carcasses, as detailed in Table 1. This process took eleven days to complete.

Table 1. Description of the meat pH measurements at the slaughterhouse

Date	pH measurement in chuck region	pH measurement in loin region	Carcasses total
20/02/2024	12	12	12
21/02/2024	7	7	
22/02/2024	7	7	
23/02/2024	13	13	13
26/02/2024	-	13	
27/02/2024	13	13	13
28/02/2024	9	9	
29/02/2024	9	9	
01/03/2024	11	11	11
4/03/2024	-	11	
12/03/2024	12	12	12
13/03/2024	9	9	
14/03/2024	9	9	
TOTAL	111	135	61

Following the collection of the aforementioned data, an analysis was conducted in Microsoft Excel 365 and Epitools Epidemiological Calculators by Ausvet (available at: http://epitools.ausvet.com.au.). This was undertaken with the objective of identifying potential associations between the variables and establishing a predictive equation for the meat pH at 48 hours post-mortem.

The subsequent stage of the study involved the visitation and characterisation of nineteen Barrosã bovine farms, with the application of the previously created questionnaire.

The final stage of the study involved accompanying two transports of Barrosã calves from their origin farms in the Barroso and Minho regions to the slaughterhouse. The transportation questionnaire, initially created, was then applied.

During this period, data from the Associação dos Criadores de Bovinos da Raça Barrosã, AMIBA (Barrosã breeders' association), was analysed in order to characterise the Barrosã Protected Designation of Origin (PDO) production system. Furthermore, the Barrosã meat pH results from the previous five years were analysed in order to characterise each farm and its productive results.

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
AI	Artificial Insemination
AMIBA	Associação dos Criadores de Bovinos da Raça Barrosã
AR	Attributable risk
ATP	Adenosine triphosphate
AVP	Arginine vasopressin
BRS	Bovine respiratory syncytial
BVD	Bovine viral diarrhea
CAPOLIB	Cooperativa Agro Rural de Boticas
CI	Confidence Interval
CRH	Corticotropin release hormone
DFD	Dark, firm, dry
e.g.	*Exempli gratia*
FP	False positive
HPA	Hypothalamic-pituitary-adrenal axis
IBR	Infectious Bovine Rhinotracheitis
i.e.	*Id est*
kg	Kilogram
km	Kilometres
m^2	Square meter
mBAS	Maternal Bovine Appeasing Substance
PDO	Protected Designation of Origin
PI3	Bovine Parainfluenza-3
PPV	Predictive Positive Value
SNS	Sympathetic nervous system
SPA	Sympathoadrenal axis
WOAH	World Organisation for Animal Health
°C	Celsius degrees

1. INTRODUCTION

1.1 Animal Welfare

According to the World Organisation for Animal Health (WOAH), animal welfare is defined as "the physical and mental state of an animal in relation to the conditions in which it lives and dies."(WOAH, 2024).

1.2 Stress and Stress Response

The behavioural needs or instinctive drives of animals, such as the need for exercise, social interaction, or control over environmental stimuli, are well-documented and have considerable importance. It is therefore important to recognise that the inability of livestock to perform such behaviours has important and serious implications (Friend, 1991).

Every day, livestock, namely bovine, are exposed to stressors, which are factors that can influence the homeostasis state, triggering an inflammatory response induced by stress. This can negatively impact animals' health, performance, welfare and overall productivity in beef cattle systems (Cappellozza & Marques, 2021). These stress stimuli can be classified as follows: psychological stress, which may be experienced upon arrival at a novel environment, during weaning or mixing distinct groups of animals; physical stress, which may result from castration, bruises caused by fights, vaccination abscesses or dehorning; and physiological stress, which may include metabolic and endocrine alterations resulting from psychological and physical stressors (Cappellozza & Marques, 2021).

As a breakdown of the animal's homeostasis is the defining characteristic of stress, the stress response is a set of physiologic mechanisms which, intuitively, is designed to reset the state of homeostasis (Buckham-Sporer et al., 2023). Mitchell et al. (1988) identified two phases of the stress response: the hypothalamic-pituitary-adrenal (HPA) axis, which is associated with environmental stressors such as noise, and the sympathoadrenal (SPA) axis, which is associated with neurogenic stressors, such as transportation (Mitchell et al., 1988).

The HPA axis is a neuroendocrine system that involves interactions between hypothalamus, pituitary gland and adrenal glands. The hypothalamus releases corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) into the hypothalamic-hypophysial portal system. This leads to adrenocorticotropic hormone (ACTH) synthesis by the anterior pituitary's corticotropic cells. ACTH then stimulates the adrenal cortex, releasing glucocorticoids, including cortisol, which facilitates the fight or flight response. Cortisol can be reliably detected in blood, hair, saliva, feces, urine, lacrimal fluid or seminal plasma (Koenneker et al., 2023; Kumar et al., 2023).

The SPA axis is comprised of the sympathetic nervous system (SNS) and the adrenal medulla. Acetylcholine, a neurotransmitter released by the preganglionic neurons of the SNS, stimulates postganglionic neurons to release noradrenaline into the target tissues, thereby activating the adrenergic receptors. Furthermore, the chromaffin cells in the adrenal medulla are also stimulated by acetylcholine, which results in the synthesis of catecholamines (dopamine, adrenaline, and noradrenaline) and their subsequent release into the peripheral blood flow (Kumar et al., 2023). These catecholamines circulate through the body and interact with α- and β-adrenergic receptors, which are expressed in numerous organs.

Consequently, the concentration of catecholamines (adrenaline and noradrenaline) and glucocorticoids (cortisol) in the blood can be utilised as an indicator of the endocrine activity in stress responses. Other physiological indicators of stress in cattle are the elevated heart rate (Buckham-Sporer et al., 2023) and the changes in milk production (Koenneker et al., 2023).

Each animal responds to a stressor in an individual manner, which is determined by genetics and past and present environmental factors, such as nutrition, social environment, and previous experiences (Friend, 1991; Mounier et al., 2006).

1.3 Farm Management and Stress

As previously stated, past experiences have been demonstrated to influence the responses of animals to handling and other stressors (Friend, 1991; Mounier et al., 2006). Mounier et al. (2006) concluded that the farmer's awareness of the sensitivity of bulls to humans or to feeding schedules and the absence of a positive attitude toward close contact with bulls are factors that limit the animals' stress and has consequences on meat pH.

The housing system and management practices of farm animals should be optimized, in order to improve their well-being, which not only goes in accordance with the consumers' interests but also is in the best interest of farmers, as it is proven to improve animals' productivity (Koenneker et al., 2023).

The attitudes of farmers toward animals and their social interactions, particularly if animals are known to each other from birth, can influence the stress levels of the animals and reduce their arousal during stressful situations. Conversely, the transport and slaughter process can represent a greater contrast for animals that are used to a quiet and regular environment on their origin farm, and therefore experience more stress during these events (Mounier et al., 2006).

The diet of the animals in question can also have an impact on the depletion of their glycogen reserves. It has been observed that a highly energetic diet appears to minimise this depletion.

Furthermore, animals that are fed before transfer are more able to withstand the physical aspects of transportation (Mounier et al., 2006).

1.4 Transportation Stress

Cattle are typically transported between the farm and the slaughterhouse for food production purposes (Nicolaisen et al., 2023). This is commonly accomplished by road, although animals may also be transported by ship or by rail in conjunction with a road journey (Buckham-Sporer et al., 2023). During the transfer to slaughter, animals experience a number of events that can lead to physical and psychological stress (Mounier et al., 2006).

The term 'transport' is used to refer to the entire process from loading the first animal on the farm to unloading the last animal at the destination (Nicolaisen et al., 2023). The European Commission published Regulation (EC) No. 1/2005 with the aim of regulating the transport of animals in an economic context and setting out the required conditions. One of those conditions is that animals' transport is only permitted if they are fit for transport and that "no person shall transport animals or cause animals to be transported in a way likely to cause injury or undue suffering to them" (Council of the European Union, 2004). Nevertheless, animal transport may introduce a multitude of physical and physiological stressors, including noise, extreme temperatures, overcrowding, commingling with unfamiliar animals, food and water deprivation, handling by unfamiliar humans and being placed in a novel environment at the destination (Buckham-Sporer et al., 2023).

The loading of animals for transport is facilitated if the farm is well-equipped, including having a loading ramp to the truck (Grandin, 1990; Mounier et al., 2006).

During the transportation and arrival of animals to the slaughterhouse, the primary hazards are physical in nature, including lengthy and rough journeys, food deprivation, inappropriate temperatures, air or water quality, aggressive interactions between animals, stockpersons or equipment. All of these can result in fatigue, hunger, thirst, thermal and respiratory discomfort, fear and pain. Furthermore, animals may also experience psychological stress, such as social disturbance or fear of unfamiliar environments, handling, novelty, loud noises, or odours (Mounier et al., 2006; Nielsen et al., 2020).

A multitude of factors influence the stress experienced by animals during transportation. These include the driving style, road conditions, the handling of animals at any stage of the journey, including loading, transportation and unloading the animals at the abattoir, route planning, vehicle design and other factors (Nicolaisen et al., 2023). Mounier et al. (2006) identified a number of physical factors that increase stress and impact meat quality by limiting the meat pH decline. These include the absence of loading facilities on the farm, transport on warm days, and a short waiting time at lairage.

They also found that the presence of animals from the same finishing group limited stress and improved the meat pH decline (Mounier et al., 2006).

During transportation, cattle experience stress, which results in alteration to protein and energy metabolism, as well as alterations to growth performance and feed intake. These alterations can manifest as weight loss, which can be attributed to factors such as gut fill loss, urination, dehydration, and long periods of fasting (Buckham-Sporer et al., 2023).

In addition, transport can affect carcass yield and meat quality. Prolonged fasting and the physical stress of transport, as well as social re-grouping at the abattoir, contribute to exhaustion and reduction in muscle glycogen concentration. Muscle glycogen is required for conversion to lactic acid, which lowers the pH of the meat after slaughter. Exhausted animals with reduced muscle glycogen before slaughter are associated with high meat pH (>5.8), due to the diminished glycogen availability for the glycolysis process, which subsequently results in reduced lactic acid production, thereby limiting the acidification of the meat. The higher meat pH values allow bacterial proliferation and reduces product shelf-life. At the same time, higher meat pH is also associated with the occurrence of "dark cutting" or "dark, firm, dry" (DFD) meat, which is unappealing to consumers (Buckham-Sporer et al., 2023; Mounier et al., n.d.).

Mounier et al. (2006) found that unloading at the abattoir can be difficult after long journeys and when animals are mixed immediately before being loaded onto the truck for transport, and that the meat pH was higher the higher temperatures were when the truck arrived at the slaughter plant. This can be explained by the fact that a short journey and heat are likely to make the animals more restless in the lorry and more willing to get out of the truck at the abattoir, making unloading easier (Mounier et al., 2006).

1.5 Stress in Lairage

As well as being a reservoir for animals, the lairage can be an opportunity for animals to recover and rest from the hazards of handling and transport to the slaughterhouse. But this only happens if there is enough space, adequate ventilation, a quiet environment, and a water supply. This means that if lairage conditions are inadequate, they can negatively impact the welfare of the cattle, either through their own effects or by aggravating the consequences of previous hazards to which cattle have been exposed to (Nielsen et al., 2020).

Typically, there is a mix of unfamiliar animals in the lairage pens, i.e. animals that were not pen-mates during rearing or fattening. As cattle are highly social animals, that naturally live in groups with social hierarchies established over time, these changes to the group usually result in social stress, with aggressive behaviour, fighting and mounting in cattle (Nielsen et al., 2020).

Lairage can be a moment to replenish glycogen resources and to recover from physical exhaustion, even if the animals are not fed, and therefore the meat pH can be lower when the waiting time at lairage is longer, but this recovery does not occur when unfamiliar animals are mixed (Mounier et al., 2006).

1.6 Moving animals to the stunning point

As cattle are gregarious animals, they feel secure in groups of animals and stressed when isolated, therefore it is recommended that they are moved in small groups, maintaining a continuous movement of animals and the subsequent groups have sight of cattle moving ahead (Nielsen et al., 2020).

Other factors that can contribute to animal welfare issues in slaughterhouses include distractions that impede the animals' ability to move freely, such as i) lighting problems (either too dim or too bright); ii) air hissing or high-pitched noise iii) air drafts blowing down the race towards approaching animals; iv) seeing movement or sparking reflections (Grandin, 1996).

Furthermore, it is essential that operators who handle cattle are cognizant of the fundamental behavioural principles of moving cattle. To facilitate the animal's forward movement, the operator should be situated within the animal's flight zone and behind the point of balance, which is commonly referred to as the shoulder of the animal (Nielsen et al., 2020).

It is therefore important to ensure that these aspects are taken into account in order to minimise the stress experienced by the animals during the transportation to the stunning point. These issues can be mitigated by ensuring that operators are adequately trained in the appropriate behaviour and positioning when moving the animals, as well as the design of the raceway (Nielsen et al., 2020).

1.7 Stunning and bleeding

Stunning is the process whereby animals lose consciousness and sensibility without pain. At this stage, animals are restrained in order to facilitate the effective stunning and killing process. The stunning process can be carried out in one of two ways: mechanical or electrical methods. Mechanical stunning methods include penetrative captive bolt, non-penetrative captive bolt, and free projectiles on the animal's skull. Electrical stunning methods include head-only and head-to-body electrical stunning (Nielsen et al., 2020).

In this phase, animal welfare consequences are manifested by pain and fear during restraint, which are caused by ineffective stunning or delayed sticking. This leads to the return to a conscious state during hoisting, sticking, and bleeding (Nielsen et al., 2020).

Following stunning, there is bleeding of cattle, which is intended to cause death in unconscious animals. It is anticipated that stunning will result in an unconsciousness that persists for a longer duration than the time elapsed between the conclusion of stunning and death due to blood loss subsequent to sticking (Nielsen et al., 2020).

If the animal is conscious at the time of sticking, cutting, or bleeding, two major welfare concerns must be considered. Firstly, the incision made in the neck activates nociceptors, inducing animals to experience pain. Secondly, death from sticking is not immediate, and the animal may experience pain, fear, and distress during a period of time (Nielsen et al., 2020).

As suggested by Nielsen et al. (2020) sticking should be performed as soon as possible, with a maximum of 60 seconds when the penetrative captive-bolt method is employed and 30 seconds for the non-penetrative captive-bolt method once the animal has been effectively stunned.

The major hazards associated with bleeding are pain, fear and distress, which may result when cattle are ineffectively stunned or recover consciousness during sticking. These issues may be attributed to a lack of adequately trained personnel and the absence of monitoring for consciousness during the sticking procedure (Nielsen et al., 2020).

1.8 From muscle to meat

The post-mortem period is characterised by a complex sequence of changes in the muscle. Following exsanguination and subsequent anoxia, skeletal muscle fibres continue to synthesise and utilise adenosine triphosphate (ATP) in an attempt to maintain homeostasis. However, once all the oxygen in the muscle is depleted, glycogen and high-energy phosphate compounds in the muscle at the time of slaughter are now anaerobically metabolised to produce ATP. In comparison to the aerobic process of ATP production, the anaerobic is less efficient, resulting in a net loss of ATP. This is due to the fact that the hydrolysis of ATP exceeds its synthesis, with the consequence that muscles gradually lose their ability to generate ATP, depleting all the molecules involved. This results in the irreversible binding between myosin and actin molecules, culminating in the phenomenon of r*igor mortis*, which is characterised by muscle tension and occurs completely within 1-12 hours post-mortem, depending on the species, muscle fibre type and pre- and post-mortem conditions (Matarneh et al., 2017).

The resolution of r*igor mortis* occurs during post-mortem storage (ageing), as a result of the loss of muscle structural integrity due to the proteolytic degradation of cytoskeletal proteins (Matarneh et al., 2017).

Another change occurring in the muscle post-mortem is acidification. This phenomenon is due to glycolysis and ATP hydrolysis, which result in the accumulation of lactate and hydrogen ions (H^+) in the muscles, leading to a gradual pH decrease from approximately 7.2 to values between 5.5 and 5.7. Values above this range enable the proliferation of proteolytic bacteria and compromise the organoleptic characteristics of the meat. The rate and extent of this decline is dependent on environmental conditions and pre- and post-slaughter handling, and it affects meat quality attributes, including colour, texture, and water-holding capacity. Furthermore, less glycogen stored in the muscle at the time of slaughter is related to higher ultimate meat pH due to a lack of sufficient glycogen for the glycolysis process and the subsequent lower quantity of lactic acid and H^+ ions produced in anaerobic post-mortem conditions (Matarneh et al., 2017).

1.9 Characterization of Barrosã breed

The Barrosã breed represents a significant cultural and historical heritage of Portuguese cattle, originally derived from mountainous regions in the northernmost region of Portugal. The geographical limits of the breed are defined by the municipalities of Montalegre, Boticas, Cabeceiras de Basto, Fafe, Guimarães and a line between Braga, Monção and Melgaço (AMIBA, 1997). This area, known as the Alto Minho and the Barroso region, is characterised by its unique agroclimatic and geomorphological conditions, which have historically challenged big agriculture explorations, in part because of the rigorous winters and the mountainous environment that restricts its fertility and the extension of the cultivable land (AMIBA, 2001). Initially, this breed played a pivotal role in the rural environment, as the utilisation of these animals, which were fully adapted to the environment, was the sole means of cultivating small land plots and terraces characteristic of the region. Furthermore, the animals enabled the natural fertilisation of poor soils and the enhancement of scarce alimentary resources, such as *Baccharis trimera* and *Ulex spp.* (AMIBA, 2001).

In the nineteenth century, the breeding of these animals was oriented towards the commercialisation of young animals for the beef industry. Additionally, farmers utilised the strength of these animals to supplement manual labour for their crop production and transport activities. Dairy production was a less significant activity for this breed (Dias & Maia, 2020).

Over time, additional breeds were introduced to the Barroso region, including the Maronesa and Mirandesa breeds, which were distinguished by enhanced work ability. This led to the development of crossbreeding programs, particularly between the Barrosã and Mirandesa breeds. Furthermore, the mechanisation of agricultural activities, the replacement of the Barrosã breed with superior dairy ability and more corpulent animals (e.g., the Minhota breed) and the ageing of the Barroso region population have contributed to a decline in the number of Barrosã animals, with only 7500 breeding females remaining in the country in 2016 (FAO, 2018).

The Barrosã breed is distinguished by its rustic appearance, well-developed anterior third and its temper. These characteristics make it well-suited for the demographic region in question and suitable for use as work animals in agriculture (Dias & Maia, 2020). The breed's horns, small but harmonious stature, and renowned meat production set it apart from other breeds (AMIBA, 1997) (Annexe D).

Currently, animal production represents the primary economic activity of the region, with extensive cattle breeding for beef, representing the dominant sector. The animal's feeding is based on natural pastures (scrubland) and permanent meadows (marches). The traditional production system is characterised by an extensive regime that is adapted to the climate. This involves animals remaining outside semi-free for the majority of the year and returning to the cowshed only at night during the colder winter months and in the case of suckling cows and their offspring (FAO, 2018).

With its high capacity for producing meat with recognised organoleptic quality, the native breed Barrosã meat was granted Protected Designation of Origin (PDO) status. Calves are slaughtered at five to nine months of age at a minimum weight of 70 kg. The dairy industry is less significant in this context, with milk usually used to feed calves (Dias & Maia, 2020).

1.10 The problem and the objective

In recent years, there has been a significant issue with the pH levels of Barrosã PDO meat, with a considerable number of carcasses exhibiting a pH of 5,8 or above. The meat quality is dependent upon a multitude of interrelated factors, in addition to the metabolic changes and post-mortem pH decline (Ouali et al., 2006). It is well established that elevated pH values result in muscle fibres being tightly packed, with the surface scattering lower light. This phenomenon leads to muscle proteins retaining more water and appearing darker. It has been demonstrated that pH values above 5,9 are associated with an increased incidence of DFD meat or dark-cutting beef, which is characterised by a higher water-holding capacity and tenderness. Furthermore, the flavour and shelf life of DFD meat are negatively affected by higher pH meat (Kumar et al., 2023). Therefore, meat pH is an indicator of meat quality. Consequently, the previous results of Barrosã PDO meat pH are cause for concern. It is evident that this is also an economic issue, as high-pH meat is rejected, has a shorter shelf life and impacts the organoleptic characteristics that define this meat.

To address this issue and identify potential explanations and solutions, we conducted this study, with the objective of characterising the production system, transportation and slaughter processes and identifying variables that possibly explain the high incidence of high meat pH values.

2. MATERIALS AND METHODS

2.1 Questionnaires

In order to collect data for this study, three questionnaires[1] were created with the aim of characterising and evaluating the work at the slaughterhouse, the farms, and the transport from the farms to the slaughterhouse. The questionnaires were developed following a review of several studies on the subject matter (Buckham-Sporer et al., 2023; Direção Geral De Alimentação E Veterinária, n.d.; Grandin, 1995, 1996; Gruber et al., 2010; Holman et al., 2021; Knowles, 1999; Mounier et al., n.d.; Nicolaisen et al., 2023; World Organisation for Animal Health (WOAH), 2023, 2024) with consultation of the relevant parties, involved, namely the *Cooperativa Agro Rural de Boticas* (CAPOLIB), the *Associação dos Criadores de Bovinos da Raça Barrosã* (AMIBA) and the Slaughterhouse *PEC Nordeste*.

2.2 Data collected

At Slaughter. The research commenced with a period of thirteen days with observation and characterisation of the work at the PEC Nordeste slaughterhouse in Penafiel, Portugal, where the Portuguese bovine breed, Barrosã, was slaughtered in accordance with the producer's organisational procedures. During this time, the questionnaire pertaining to slaughterhouse work was administered.

The initial step of the questionnaire involved an assessment of the animals and the lairage conditions. On the day preceding the slaughter, the animals were transported from their respective farms of origin to the slaughterhouse. Those transported together were then housed in the same pen until slaughter. A total of five pens of animals were assessed, corresponding to five different transports and five assessment days. A questionnaire was administered on each assessment day.

The subsequent stage of the process involved the assessment of the movement of animals to the stunning area, followed by the evaluation of the stunning and bleeding of each animal.

The final step was to measure the pH of each carcass using a portable meat pH and temperature meter, HI99163, HANNA instruments® (Figure 1), which had been calibrated with 4.01 and 7.01 pH solutions.

[1] In view of the restricted number of pages in this work, the questionnaires are not included. However, they are available for consultation upon request to the author.

Figure 1. Portable meat pH and temperature meter HI99163, HANNA instruments® used to measure the pH of the Barrosã DOP meat at the slaughterhouse PEC Nordeste, in Penafiel, Porto.

The slaughter of the animals for this study occurred on Tuesdays and Fridays. The pH measurements were taken at 0, 24 and 48 hours post-mortem in each carcass for Tuesday's slaughter and at 0 and 72 hours post-mortem in each carcass for Friday's slaughter. This difference in the measurement timing was due to the schedule of the work at the slaughter plant. The pH was measured in two distinct regions for each carcass: the chuck region and the loin region. The pH meter was inserted into the carcasses at a comparable depth and location. The muscle *longissimus dorsi* is typically employed to ascertain the ultimate pH and thus, evaluate the quality of the carcass (Mounier et al., 2006). A total of 246 pH measurements were conducted, with 111 in the chuck region and 135 in the loin region. This was done on 61 carcasses, as indicated in Table 1.

The predictive positive value (PPV) of the pH measurement at 24 hours post-mortem is defined as the value of the prediction of the ultimate meat pH measurement (at 48 hours post-mortem) based on the meat pH value at 24 hours post-mortem. The PPV was calculated by dividing the number of problematic meat pH results at 48 hours post-mortem by the number of problematic meat pH results at 24 hours post-mortem. A false positive (FP) result was defined as a carcass that exhibited a meat pH problem at 24 hours post-mortem, but not at 48 hours.

On Farms. Twenty-two farms were selected by the technical officers of the breeder's organisations following a purposive sampling procedure, which was based on the problem under study and the assessment of the experts. Farms were divided into two groups: those with good results; and those with problematic results. Each category had eleven farms attributed. This selection was made based on the meat pH results from May 2020 to May 2024. Farms with problematic results were defined as those with more than five carcass pH results of 5.8 or above, while farms with good results were defined as those with five or fewer carcass pH results of 5.8 or above. Following this selection, visits were conducted to the farms, interviews were conducted with the farmers and the previously created questionnaire was applied. Additionally, observations were made of the animals and farm conditions. Only nineteen of the twenty-two selected farms were assessed, nine farms were from the group with good results, defined as the Control Group, and ten from the group with problematic results, defined as the Problem Group. This process took four days, and nineteen questionnaires were applied. The farms that were visited and assessed were distributed throughout the Barroso and Minho regions, as illustrated in Figure 2, in order to provide an overview of the diverse characteristics and production systems present in different municipalities.

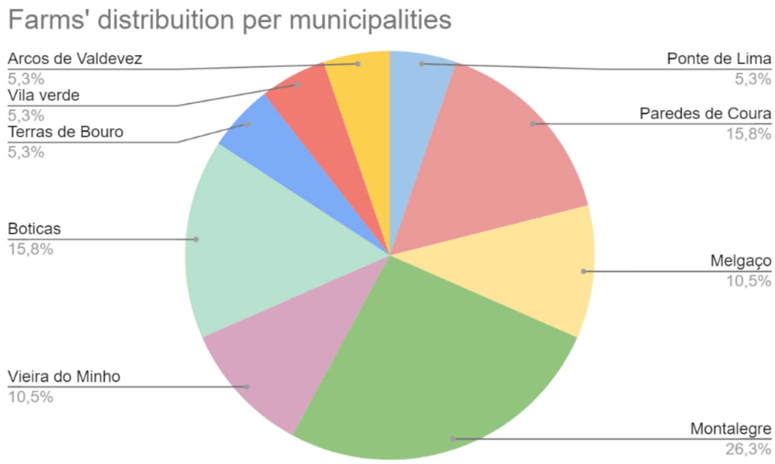


Figure 2. Distribution per municipalities of the farms assessed (N=19).

A statistical analysis was conducted on the previous meat pH results obtained from the animals slaughtered from the assessed farms from May 2020 to May 2024, using Microsoft Excel 365 and Epitools Epidemiological Calculators by Ausvet. The proportion of problematic pH results in relation to the total number of pH results was calculated for each farm and each group by dividing the number of carcasses with problematic results (pH ≥ 5.8) by the total number of carcasses.

The questionnaire sought to ascertain nutrition, weaning timing, water availability, number of animals (calves, adult females and adult males) per farm, calves' space allowance, animal management, vaccination, deworming, disease, culling and mortality rates, mean body condition score, mean behavioural score and breeding method employed. Questionnaires' results were statistically analysed and T-tests were performed, using a significance level (α) of 5%.

During transport. A study was conducted to assess the transportation of animals from their origin farms to the slaughterhouse. A questionnaire was administered to ascertain the conditions of the transportation. Two transports were accompanied, with a total of twenty-seven animals transported. One of the transports accompanied was from the municipalities of Boticas and Montalegre (Barroso region) to the slaughterhouse, in Penafiel, Porto and the other one was from the Minho region to the same slaughterhouse. The pH of the meat of these carcasses was measured and recorded by the food engineers working at the slaughterhouse, in accordance with standard practice.

2.3 AMIBA's Database

A statistical analysis was conducted on the AMIBA, Associação dos Criadores de Bovinos da Raça Barrosã, database to ascertain the status of the Barrosã production system at the time of the study (May 2024). This entailed an analysis of the number of animals, farms, farmers, animals per age group, and the farms' geographic distribution. The data were analysed using the Microsoft Excel 365 and Epitools Epidemiological Calculators by Ausvet.

2.4 Meat pH results of the animals slaughtered between May 2020 and May 2024

The database of CAPOLIB, Cooperativa Agro Rural de Boticas, comprehending the data from Barrosã PDO slaughtered between May 4th 2020 and May 17th 2024 at the PEC Nordeste Slaughterhouse, was analysed using Microsoft Excel 365 and Epitools Epidemiological Calculators by Ausvet. A total of 4800 Barrosã PDO calves were slaughtered during the specified period from 391 distinct farms. Of these, 1871 were females (39.0%) and 2929 were males (61%). Nevertheless, only 3978 carcasses from 371 distinct farms underwent meat pH measurement, as some of the carcasses were sold prior to the pH measurement being conducted. Of the 3978 animals, 1538 were female (38.7%) and 2440 were male (61.3%). Barrosã PDO slaughters occurred on Tuesdays and Fridays, in accordance with the Slaughterhouse work schedule. The pH value of the carcasses was determined by food engineers working at the slaughterhouse approximately 24 or 72 hours post-mortem, depending on whether the slaughter occurred on a Tuesday or on a Friday, respectively. 1539 measurements were done 24 hours post-mortem while the remaining 2439 were done 72 hours post-mortem. The measurements were done using a portable meat pH and temperature meter, inserted into the loin region at comparable depth and location for each carcass. Furthermore, the meat pH results were divided into four categories (<5.8; [5.8; 5.9[; [5.9; 6.0[and ≥6.0) and separated by animals' sex. The relation between sex and meat pH category, and the proportion of each pH category within each sex were studied using Microsoft Excel 365 Epitools Epidemiological Calculators by Ausvet, and Z-tests were performed, using a confidence level of 95% (significance level (α) of 5%).

In addition, animals' mean live weight per age group (months), was also analysed.

3. RESULTS

3.1 The Barrosã breed Production System

At the time of the writing (May 2024), the total number of PDO Barrosã farms registered on AMIBA is 1388, distributed across 22 municipalities. Montalegre is the municipality with the highest number of farms (276), followed by Arcos de Valdevez (251) and Vieira do Minho (167). The distribution of the number of farms per municipality is illustrated in Figure 3.

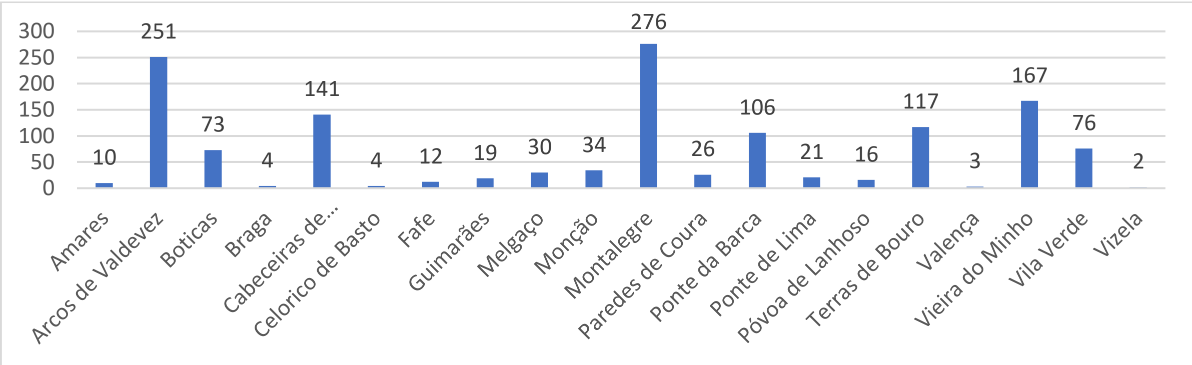


Figure 3. Distribution of the number of PDO Barrosã breed farms registered on AMIBA per municipality. (Source: AMIBA's database)

The number of adult animals of the Barrosã breed per municipality varies considerably, from 2296 animals in Montalegre to 2 animals in Vizela, as represented in Figure 4. A more complete analysis is available in Annexe A.

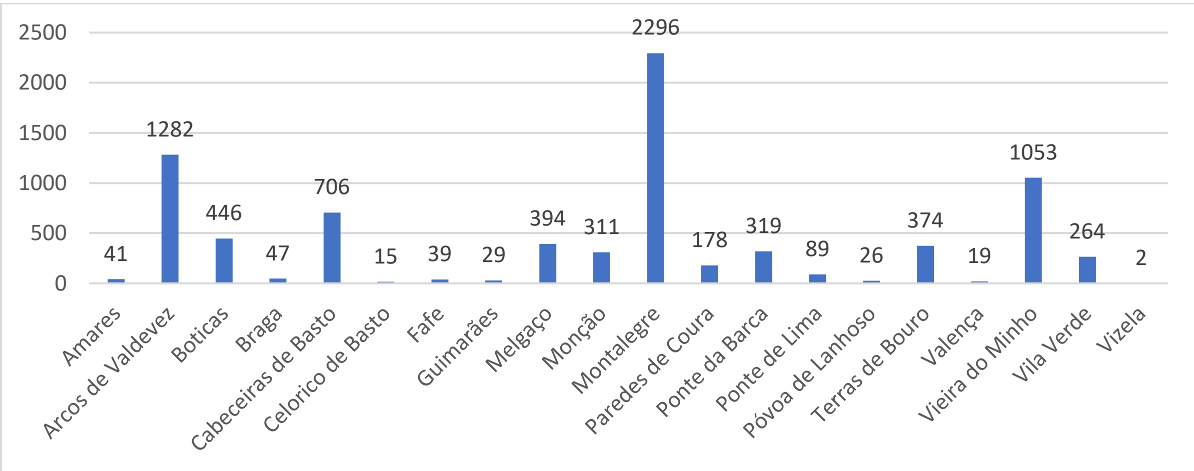


Figure 4. Distribution of the number of PDO Barrosã adult animals registered on AMIBA per municipality. (Source: AMIBA's database)

3.2 Characterization of the Production System of the sampled herds

A total of nineteen farms were visited and characterised by applying the previously created questionnaire related to the farms.

The proportion of problematic pH results in the control group (N=9) was 0.231 and in the problem group (N=10) was 0.382. The mean proportion of problematic pH results for all the farms assessed (N=19) was 0.311. The statistical analysis concluded that there was no significant difference (z-value = 0.7 and p-value = 0.478) between the proportion of problematic results in the control and in the problem groups (Table 2).

With regard to nutrition, there were some differences between the farms, but all nineteen farms fed hay to their animals and all of them had feed always available *ad libitum*. In the problem group, seven out of the ten farms assessed (70%) commenced feeding hay to calve since parturition. One farm (10%) commenced feeding hay one week after parturition, one farm (10%) commenced feeding hay at three months old and one farm (10%) commenced feeding hay at four to five months old. In the control group, five out of the nine selected farms (approximately 56%) commenced feeding hay since parturition, while four farms (approximately 44%) initiated hay feeding at three months old.

Two of the ten farms in the problem group (20%) provided silage to their animals in contrast with none of the control group farms. Four (approximately 44%) and six (60%) of the control and problem group farms, respectively, provided concentrate to their animals, and grain products were fed in all (100%) control farms and 9 (90%) of the problem farms. The questionnaire also sought to ascertain the weaning timing in relation to slaughter. The results indicated that only one farm (approximately 11%) in the control group and four farms (40%) in the problem group weaned their calves before going to slaughter at seven months and four to five months of age, respectively.

A total of four (40%) problem farms have salt blocks available to the animals, while in the control group, only one farm (approximately 11%) had salt blocks. Furthermore, seven of the nine control farms (approximately 78%) reported occasionally feeding potatoes or potato peels to their animals. Of these seven control farms, one (approximately 11%) occasionally feeds rye soaked in wine, while another (approximately 11%) feeds turnip, in addition to the potatoes.

The questionnaire also sought to ascertain whether water was consistently available to the animals. Only two control farms (approximately 22%) did not have water freely available, and one control farm (approximately 11%) provided water *ad libitum* only to the weaned calves.

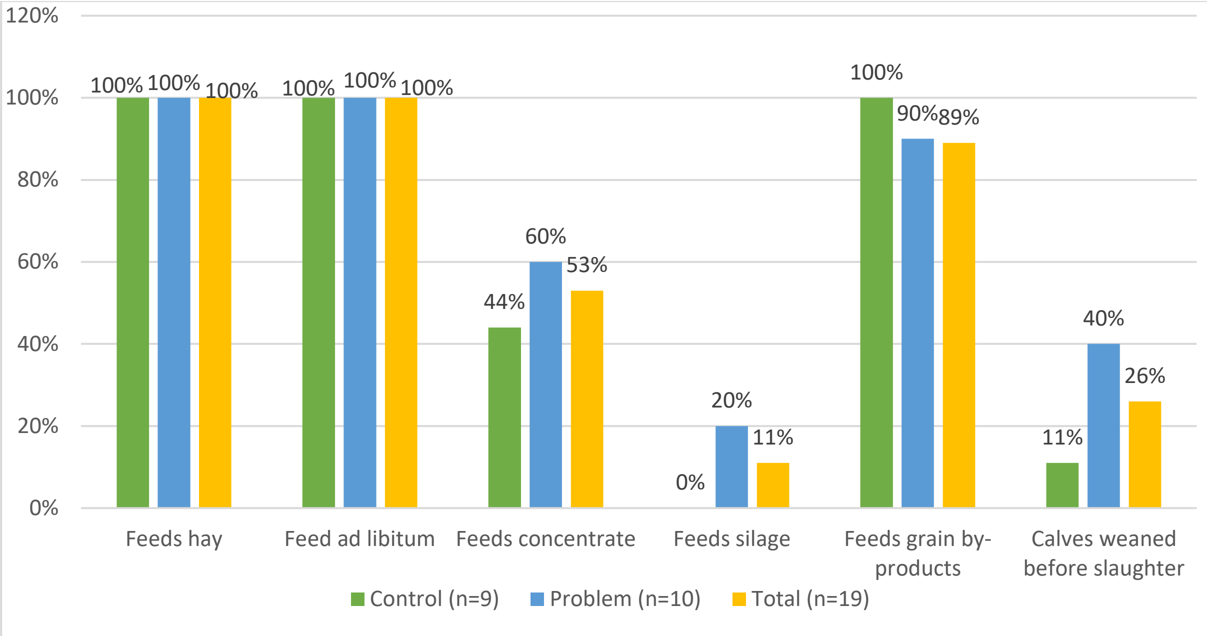


Figure 5. Description of the questionnaires' results regarding nutrition applied to nineteen Barrosã PDO farms (n=19), divided into the control (n=9) and problem groups (n=10).

The number of animals per farm (farm size) was assessed, with a distinction made between the number of adult males and females, as well as calves. The results are presented in Figure 6. The statistical analysis concluded that there was a significant difference (p-value = 0.012) between the farm size of the control and the problem groups (Table 2).

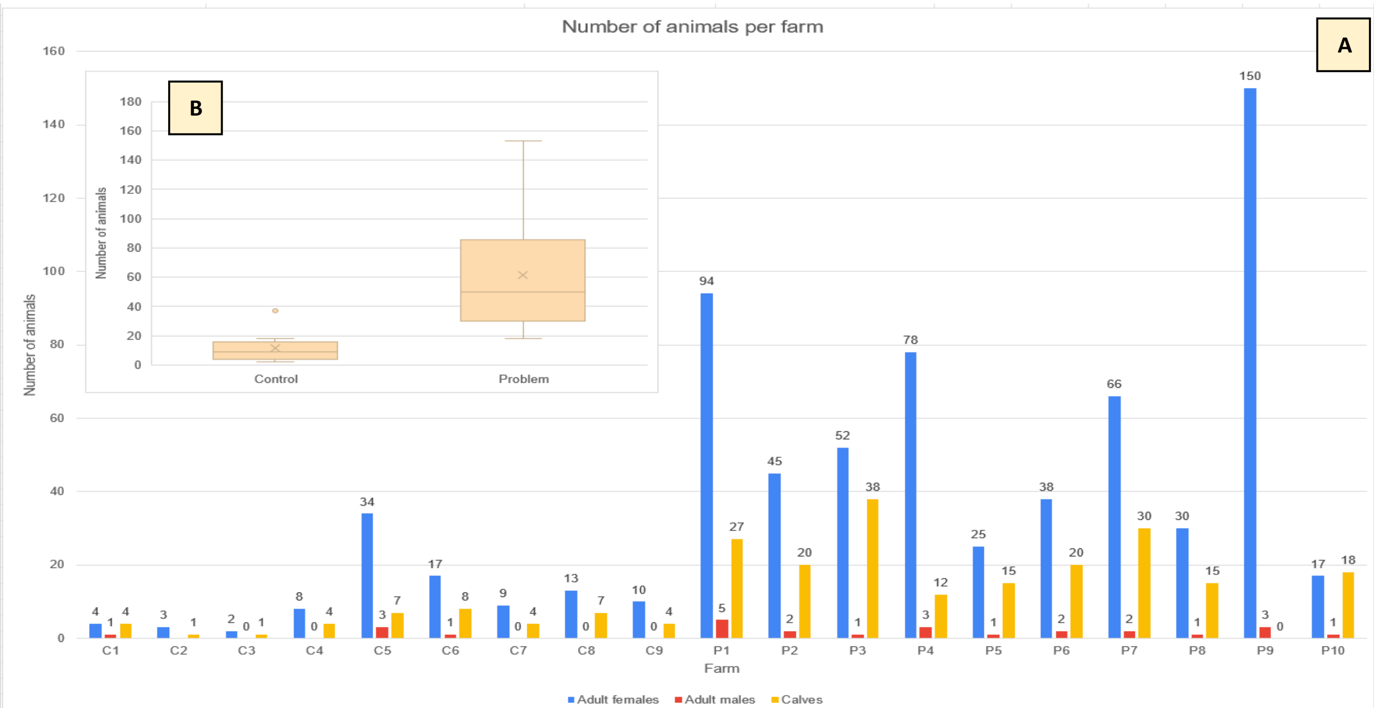


Figure 6. (A) The number of animals, discriminating between adult females, adult males and calves, of the nineteen farms assessed (n=19), divided into the control (n=9) and problem groups (=10), and (B) the mean number of adult animals per farm (i.e. farm size) in the control (n=9) and problem groups (n=10).

With regard to animal management, there were notable differences between farms assessed. It is noteworthy that none of the control farms send calves to the pasture with their mothers. Instead, calves are kept stabled and the mothers return at the end of the day to the stable to feed the calves. In the problem group, only two farms (20%) do not send their calves to the pasture. Three farms (30%) send their calves to the pasture at any age and five farms (50%) send their calves to the pasture until they are four up to six months old.

In five control farms (approximately 55%), animals are stabled separately by age. This occurs in four farms (40%) in the problem farms. In the control group, five farms (approximately 55%) have shared pens for mothers and calves, while four farms (approximately 44%) have individual pens for each calf and its mother. In contrast, none of the problem farms have individual pens, with animals instead being housed together.

Concerning vaccination in the problem group, one farm (10%) does not vaccinate its animals, one farm (10%) vaccinates against IBR, Bluetongue disease, and Anthrax, and one farm (10%) vaccinates against IBR, PI3, BVD and BRS viruses. The remaining seven problem farms vaccinate once a year in accordance with the Sanitary Plan for the region. In the control group, all farms (100%) vaccinate according to the Sanitary Plan of the region.

Concerning deworming, all the control farms use ivermectin once a year for adult cows. One control farm (approximately 11%) uses ivermectin once a year for adult cows and calves over 2 months old. In the problem group, one farm (10%) uses ivermectin twice a year for adult cows only, four farms (40%) deworm their adult cows twice a year and their calves once, one farm (10%) deworms their adult cows and calves once a year and four farms (40%) deworm only their adult cows once a year.

Disease incidence was also assessed in the questionnaire. In the problem group, one farm (10%) had some animals affected with mucosal disease in the last three years, one farm (10%) had one calf with suspected Anthrax disease in 2023 and one farm (10%) had two animals affected with Tuberculosis in 2019. In the control group, one farm (approximately 11%) had a recent history of three cows affected by diarrhea with significant weight loss. The culling rate of the evaluated farms is minimal, with one problem farm (10%) reporting culling ten to fifteen animals per year and the remaining farms, both problem and control, reporting culling up to three animals per year or no culling at all. The primary reasons for culling were infertility, reduction in milk production, age, mammary gland problems, and behavioural issues.

The evaluated farms did not have high mortality rates. In the previous two years, in the control group, two farms (approximately 22%) registered a calf death for reasons non-determined, one farm (approximately 11%) registered one adult cow death due to an accident and one calf death for reasons non-determined, and one farm (approximately 11%) recorded two stillbirths. In the problem group, one farm (10%) registered two calves deaths caused by Diarrhea, one farm (10%) registered two heifers deaths due to an accidental fall, and one farm (10%) registered one calf death for reasons non-determined.

Additionally, the mean body condition of the animals per farm was evaluated on a scale from 1 ("Very thin") to 9 ("Very fat"), using the Barrosã Body Condition Score table (AMIBA, 2023) (Annexe B). Scoring discriminated between adult cows and calves, as their body condition is very distinct. The results are presented in Figure 7. The statistical analysis concluded that there was no significant difference (p-value = 0.988 for cows and p-value = 0.267 for calves) between the body condition score of the control and the problem groups (Table 2).

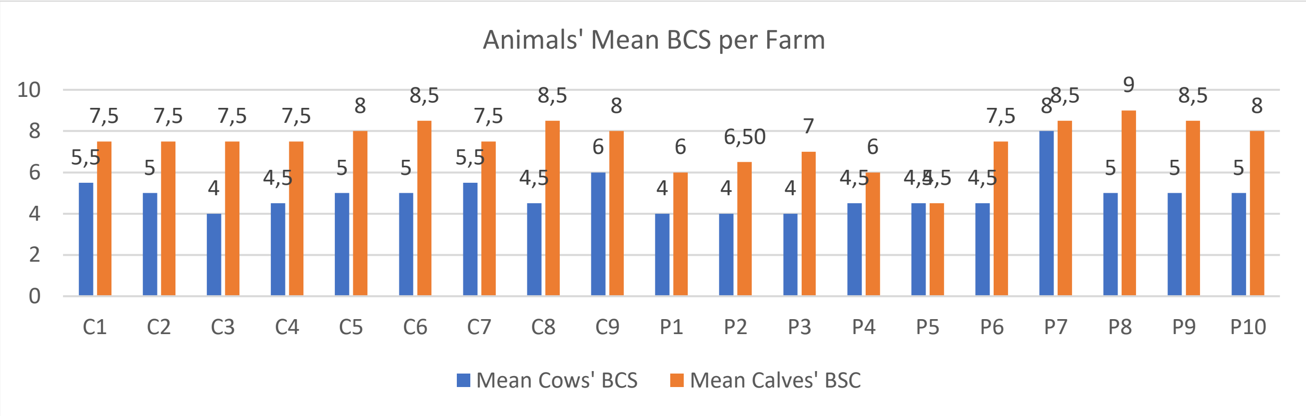


Figure 7. The mean Body Score Condition (BSC) of the animals of the nineteen Barrosã PDO farms assessed (n=19), divided into control (n=9) and problem (=10) groups. The scoring was based on the Barrosã Body Condition Score table (AMIBA, 2023) on a scale from 1 to 9, with a score of 1 being "Very thin", and a score of 9 being "Very Fat".

The results of the behaviour scoring of farms' animals are expressed in Figure 8. This classification was based on the Behaviour Scoring of the Barrosã Breed (AMIBA, 2023) on a scale from 1 to 7, with a score of 1 being an animal that is not afraid and approaches the classifier, and a score of 7 being an animal that attacks the classifier. The mean behaviour score was 2.92 (standard deviation = 0.42). The statistical analysis concluded that there was no significant difference (p-value = 0.829) between the behaviour score of the control and the problem groups (Table 2).

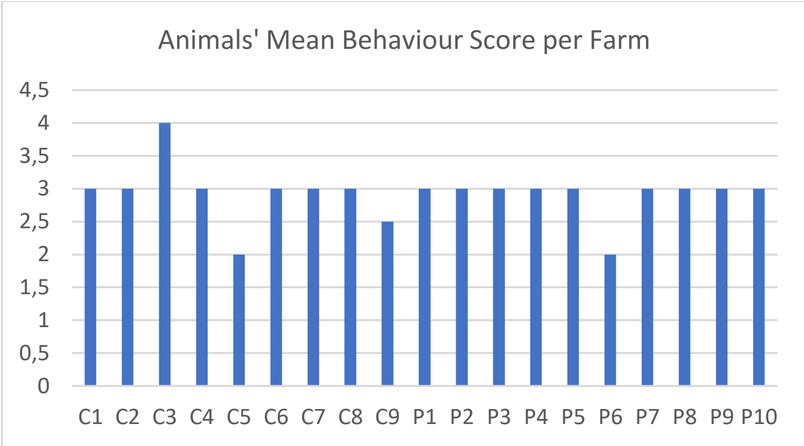


Figure 8. Animals' Mean Behaviour Score of the nineteen farms assessed (n=19) divided into control (n=9) and problem groups (n=10), based on the Behaviour Scoring of the Barrosã Breed (AMIBA, 2023). The scale used comprehended the numbers between 1 and 7, a score of 1 being an animal that is not afraid and approaches the classifier, and a score of 7 being an animal that attacks the classifier.

The breeding method employed by each farm was evaluated. The results indicated that three control farms (approximately 33%) utilized natural mating with the farm's bull, five control farms (approximately 55%) employed Artificial Insemination (AI), and one control farm (approximately 11%) utilized a combination of both methods, natural mating, and AI. All of the farms in the problem group (100%) employed natural mating with the farm's bull. Only one of the farms in the problem group (10%) conducted tests and implemented control measures to address male reproductive diseases.

Table 2. Statistical tests utilised in the study and results.

Variable	Statistical test utilised*	Control Group	Problem Group	P-value	95% CI for mean	Conclusion
The proportion of problematic pH results per group	Two Sample Z-test for difference between 2 proportions	x = 0.231 (sdev = 0.217)*2	x = 0,382 (sdev = 0.090)*2	0.478 (z-value = 0.7)	-0.2657 - 0.5677	As the test statistic value is greater than the critical value, we cannot reject the null hypothesis. Therefore, we conclude that the proportion of problematic pH results was **not significantly different** between the control and the problem groups
Mean number of adult animals per farm per group (Farm size)	Two Sample T-test for difference between 2 means	12.5 (sdev = 10.723)*2	57.4 (sdev = 40.863)*2	0.005	15.209 – 74.591	As the test statistic value is less than the critical value, we reject the null hypothesis. Therefore, we conclude that there was a **statically significant difference** between the number of adult animals per farm in the control and the problem groups
Mean Cows' Body Condition Score per group	Two Sample T-test for difference between 2 means	4.938 (sdev = 0.612)*2	4.944 (sdev = 1.180)*2	0.989	-0.910 – 0.932	As the test statistic value is greater than the critical value, we cannot reject the null hypothesis. Therefore, we conclude that there was **no statically significant difference** between the cows' mean body condition score of the control and the problem groups
Mean Calves' Body Condition Score per group	Two Sample T-test for difference between 2 means	7.875 (sdev = 0.433)*2	7.278 (sdev = 1.415)*2	0.242	-0.442 – 1.636	As the test statistic value is greater than the critical value, we cannot reject the null hypothesis. Therefore, we conclude that there was **no statically significant difference** between the calves' mean body condition score of the control and the problem groups
Mean Behaviour score per group	Two Sample T-test for difference between 2 means	2.938 (sdev = 0.527)*2	2.889 (sdev = 0.316)*2	0.807	-0.366 – 0.464	As the test statistic value is greater than the critical value, we cannot reject the null hypothesis. Therefore, we conclude that there was **no statically significant difference** between the mean behaviour score for the control and the problem groups

*Note: The critical value was calculated using a 5% significance level (α).
*2 Note: "x" represents the mean and "sdev" represents the standard deviation.

To summarize, the only studied variable that was significantly different between the control and problem groups was the mean number of adult animals per farm. All of the remaining variables (the proportion of problematic pH results, the mean cows' and calves' body condition score and the mean animals' behaviour score) did not differ significantly between the two groups.

3.3 Characterization of the Transport to the Slaughterhouse

The production system is characterized by two somewhat different regions: the Barroso region and the Minho region. Consequently, two transports were accompanied, one being from the Barroso region and the other from the Minho region. This enables the representation of both regions and their differences in this study.

Transport 1

The Transport 1 was between the Barroso region and the slaughterhouse, transferring a total of thirteen Barrosã calves from three different farms, as described in Table 3. The transport occurred in May 2024, with an outside temperature that varied between 6°C and 10°C.

Table 3. The number and age of the animals transported in the two transporters accompanied from the Barroso and Minho regions, respectively, to the slaughterhouse PEC Nordeste, in Penafiel, Porto, the time of loading and unloading at the slaughterhouse, and the total duration of the transport in hours and kilometres.

Transport	Farm	Number of animals transported	Animals' age	Loading at	Unloading at	Duration of the transport (hours)	Duration of the transport (kilometres)
1	1	1	6.5 months	11:00 am	3:40 pm	4:40	125
	2	2	8.5 months	11:30 am	3:40 pm	4:10	121
	3	10	9 months	12:14 pm	3:40 pm	3:26	96
2	4	1	9 months	2:35 pm	6:15 pm	3:40	130
	5	12	9 months	3:00 pm	6:15 pm	3:15	137
	6	1	8 months	3:00 pm	6:15 pm	3:15	137

The truck had an area of 15.12 m^2 with a separation grid that divided the space into two compartments, one with 5.04 m^2 and the other with 10.08 m^2. The three animals from farms 1 and 2 were loaded into the smaller compartment (designated Compartment A), leaving the larger compartment (designated Compartment B) for the ten animals from farm 3.

The space allowance, i.e. the floor area allocated per calf (in m^2 per head), was calculated and the results were 1.68 m^2 and 1.01 m^2 for the compartments A and B, respectively. These values (area per animal) are in accordance with the Council of the European Union (2004), which establishes the minimum space allowance in 0.40-0.70 m^2/animal for medium sized calves (110kg) and 0.70-0.95 m^2/animal for heavy claves (220kg). The stocking density, which describes the weight of the animal per unit floor area, expressed in kilograms (kg) per square meter (m^2), was 153.80 kg/m^2 and 232.14 kg/m^2 for the compartment A and B, respectively. Finally, the stocking rate, expressed in heads per unit floor area (m^2), was approximately 0.60 heads/m^2 and 0.99 heads/m^2 for the compartment A and B, respectively.

It should be noted that although not all the animals were of the same age, they were almost the same size, apart from the animal from farm 1, which was slightly shorter than the rest.

The loading period, i.e. the interval between the arrival and departure of the truck from the farm, recorded was two, ten, and ten minutes for farms 1, 2, and 3, respectively.

The behaviours of the animals during loading were observed and recorded:

- in Farm 1, the animal exhibited signs of mild distress and attempted to flee once, jumping against a wall. However, he subsequently proceeded to ascend the ramp to the truck without resistance.
- in Farm 2, the animals exhibited signs of nervousness and restlessness and engaged in a minor altercation with older animals on their way to the truck. The environment was characterised by a high level of noise, with the individuals conducting the animals yelling.
- in Farm 3, the group of animals was larger (ten calves) and initially exhibited signs of slight nervousness and agitation. However, upon being conducted to the truck ramp, all animals displayed calm behaviour and did not attempt to escape. The animals proceeded to the truck smoothly and without any resistance.

The truck lacked both a cooling and a warming system, as well as a thermometer, rendering the temperature during the transport unrecorded. Nevertheless, the outside temperature on that day ranged between 6°C and 10°C.

The duration of the transport was 4 hours and 40 minutes, 4 hours and 10 minutes, and 3 hours and 26 minutes, as represented in Table 3. This included a stop of 1 hour and 8 minutes where animals were not unloaded. During the transport, the animals were not offered any water or food. However, as soon as they arrived at the slaughterhouse, they had access to freshwater continuously.

The driver was experienced and demonstrated a smooth, careful, and attentive driving style, despite the challenging road conditions encountered during the transport. These included numerous potholes and bumps, as well as tight curves, which are typical of the Barroso and Minho regions.

Upon arrival at the slaughterhouse, none of the animals exhibited any signs of injury or distress, and appeared healthy.

The meat pH of all carcasses was below 5.8 at 72 hours post-mortem.

Transport 2

Transport 2 was conducted between the Minho region and the slaughterhouse, transferring a total of fourteen Barrosã calves from three different farms, as detailed in Table 3. The aforementioned transport occurred in May 2024, with the external temperature fluctuating between 10°C and 15°C.

This transport differed from transport 1 in that all animals from different farms were collected separately at the origin farms and then transported to a reunion point. At this point, they were all entered into a larger truck and made the final transfer to the slaughterhouse.

Calf from Farm 4 was transported to the reunion point in a truck with an area of approximately 6 m^2. Due to the geographical proximity of Farms 5 and 6, calves from those farms were transported to the reunion site together in the same vehicle with an area of approximately 15.75 m^2. At the reunion point, all animals were transferred to a vehicle with an area of approximately 15.75 m^2, which transported them to the slaughterhouse in Penafiel, Portugal. In this vehicle, there was no physical separation between animals from the different farms and ages.

The space allowance, expressed in m^2 per head, was calculated and the results were 1.13 m^2 per head. The stocking density, expressed in kg per m^2, was 193.78 kg/m^2. Finally, the stocking rate, expressed in heads per unit floor area (m^2) was approximately 0.89 heads/m^2.

The animals were approximately the same size, with the exception of the two animals from Farm 4 and Farm 6, which were slightly smaller.

The loading period, defined as the time between truck arrival and departure from the farm, was measured for Farm 4 and was found to be 5 minutes. However, it was not possible to measure the loading period for Farms 5 and 6.

The behaviour of the animals during loading was only assessed and described for Farm 4. The calf from Farm 4 displayed some nervousness and was somewhat apprehensive about entering the truck, but he then proceeded to the truck ramp voluntarily a few seconds later.

The truck lacked both a cooling and a warming system, as well as a thermometer, rendering the temperature during transport unrecorded. Nevertheless, the outside temperature on the day in question varied between 10°C and 15°C.

The duration of the transport, defined as the time between loading at the origin farm and unloading at the slaughterhouse, for animal from Farm 4 was 3 hours and 40 minutes, as described in Table 3. This included a stop of 1.5 hours at the reunion point, waiting for the arrival of animals from Farms 5 and 6. During this time animals were transferred to the vehicle transporting them to the slaughterhouse.

During the transport, the animals were not provided with water or food. However, upon arrival at the slaughterhouse, they were given access to freshwater continuously.

The transfer from Farm 4 to the reunion point involved navigating roads with somewhat poor roads conditions. Nevertheless, the driver was experienced and drove carefully and smoothly.

The transfer from Farms 5 and 6 was not accompanied.

The transport from reunion point to the slaughterhouse followed roads in good condition, mostly highways. The driving style was careful and smooth, without drastic speed changes.

At the arrival at the slaughterhouse, none of the animals presented signs of injury during the transport and appeared healthy.

The meat pH of the animals transported was measured 24 hours post-mortem. The two highest values were 5.79 and 5.80, corresponding to two of the animals from Farm 5. The remaining carcasses presented pH values of 5.76 or below.

3.4 Characterization of the work in the Slaughterhouse

In this study, the lairage time, i.e. the interval between the unloading of the animals and their slaughter, exhibited a range from 15 hours up to 18 hours and 15 minutes, with an average of 15 hours and 30 minutes. During this period, the animals were not provided with food but had access to water *ad libitum* and were housed separately from other species and other bovine breeds. Additionally, the animals in the same pen were of a similar age and size. Animals transported together in the same day were housed in the same pen, except for three animals from one transport that were housed separately due to their health status, namely suspended B3 status.

The temperature in the lairage pens varied between 7°C and 15°C, depending on the environmental temperature. The pens had sufficient space for all the animals to be standing, but in two of the five days there was not sufficient space for all animals to be lying down. In addition to the aforementioned observations, the questionnaire also included a section on injuries, locomotor problems, signs of diseases, and other noteworthy aspects observed during the lairage period. The results are presented in Table 4.

Table 4. Description of the animals' injuries, diseases or other aspects assessed pre-slaughter, in lairage and animals' behaviour assessed pre-slaughter, in lairage. Data corresponds to five assessment days, which translates to five slaughter days, with a total of 61 Barrosã calves being slaughtered at the PEC Nordeste Slaughterhouse.

Assessment Day	Number of animals assessed (n)	Description of the Injury / Disease / other aspects	Description of animals' behaviour According to AMIBA's recommendations*
1	12	**One animal lame**, with the left front leg stretched in front, avoiding supporting weight. **One animal initially laying down**, got up once the evaluator got close and a few minutes after laid down again, showing signs of weakness and tiredness	Animals were **generally curious**, moving in the direction of the evaluator and not scared of the people around. The group was classified with category **three**.
2	13	**None**	The group was classified between categories **four and five**.
3	13	One animal had diarrhea	Some animals were curious. Three of the animals were housed separately due to their health status and showed some fear and scariness, avoiding proximity with the evaluator. The group was classified with category **four**.
4	11	**None**	Animals were less curious and somewhat scared, avoiding human contact and showing some anxiety and fear. The group was classified with category **four**.
5	12	**One animal had a rope on the neck**, suggesting being tied in at the origin farm or during the transport.	Animals were less curious, moving away from humans to the opposite side of the pen. The group was classified with category **four**.

*Note: Behaviour scores were determined based on the AMIBA's behaviour classification table for the Barrosã breed (Annexe B).

None of the animals described in Table 4, with signs of injury, disease or other aspects exhibited meat pH problems (i.e. pH values of 5.8 or above).

Additionally, the behaviour of the animals was evaluated using the Barrosã breed behaviour assessment table (Annexe B) (AMIBA, 2023), with the results presented in Table 4. The average behaviour score was four, ranging from category three to five. In general, the animals displayed curiosity and exhibited minimal fear and avoidance, with some animals approaching human contact and others avoiding it and moving to the opposite side of the pen.

The conduct of animals to the stunning point was also evaluated. The race leading to the stunning point was in a straight line, without curves, and with a non-slippery cement floor. There were pneumatic gates along the race, which closed behind the animals, preventing them from retreating. Halfway through the circuit, the floor became more inclined and there were small non-slippery grooves that prevented animals from slipping. Along the race, there are rubber structures on the sides (see Annexe C) to prevent the animals from seeing people walking alongside. When the animals reach the stunning pen, a pneumatic gate closes after the animal enters, preventing the animals behind from seeing it and becoming distressed.

The animals were transferred through the race in an orderly manner, without loud noises or yelling and sometimes with the use of the electric cattle prod. No distractions were provided for the animals.

The slaughterhouse in question employed a method of mechanical stunning with a penetrating captive bolt gun aimed at the intersection of two imaginary lines drawn between the eyes and the centre of the base of the opposite horn bud. Given that Barrosã calves have a comparatively small body size in comparison with other animals slaughtered in this slaughterhouse, it was not utilised the chin lift of the stunning pen as it would be too big for them. As a consequence, the animals' heads were unsupported throughout the stunning. Following stunning, the animals were bled, with chest and neck sticking: first cutting the skin longitudinally from the neck down to the chest, following the midline and then inserting a knife into the chest near the heart. The operator then inserted a knife into the neck on both sides. The time between stunning and bleeding varied between 60 and 130 seconds, with an average of 87 seconds.

The operators were adequately trained and in sufficient numbers to perform the requisite tasks. The stunning and bleeding devices were well maintained and cleaned at the conclusion of the slaughter process.

The duration of the carcass suspension in the line, i.e. the time between bleeding and refrigeration, was also measured. This varied between fifty minutes and four hours. The latter was due to a mechanical problem in the line that required the line to stop for a long period of time, thereby increasing the duration of the carcass suspension.

Overall, the assessment of the slaughter process was considered organised and efficient.

3.5 Description of the meat pH results

The work at the slaughterhouse PEC Nordeste, in Penafiel, Portugal, was accompanied for a period of eleven days, during which time the slaughter of sixty-one bovine animals of the Barrosã

Portuguese breed was carried out. Results of measurement are presented and summarized in table 5. A total of 246 pH measurements were taken, with 111 being conducted in the chuck region and 135 in the loin region, across a total of 61 carcasses. Twelve of the sixty-one carcasses were sold by the slaughterhouse before the measurements at 24, 48, or 72 hours post-mortem, therefore only one pH measurement, right after slaughter, was taken from these twelve carcasses. Due to the work schedule of the slaughterhouse, it was not possible to measure the pH at 24 and 48 hours post-mortem on the carcasses from Friday's slaughters, i.e. slaughters 2 and 4. Instead, was conducted a measurement at 72 hours post-mortem on these carcasses.

Table 5. Mean and standard deviation of the pH results for each of the five days of slaughter accompanied at the PEC Nordeste Slaughterhouse. The results are divided by measurement timing (0 h, 24 h, 48 h, and 72 h post-mortem), and by the carcass region used for the measurement (chuck and loin region). Sixty-one Barrosã calves were slaughtered and had their meat pH measured. In total, there were made 246 pH measurements, with 111 being conducted in the chuck region and 135 in the loin region. Twelve carcasses only had one pH measurement (at 0 h post-mortem), since they were sold before the subsequent measurement at 24, 48, or 72 hours post-mortem.

Slaughter number	**Number of carcasses**	**Meat pH measured at**	**pH measured in chuck region**		**pH measured in loin region**	
			Mean	**Standard deviation**	**Mean**	**Standard deviation**
1	12	0 h post-mortem (n=12)	6.43	0.16	6.17	0.25
		24 h post-mortem (n=7)	5.85	0.12	5.72	0.09
		48 h post-mortem (n=7)	5.7	0.03	5.63	0.04
2 (Friday)	13	0 h post-mortem (n=13)	6.70	0.18	6.81	0.17
		72 h post-mortem (n=13)	Not measured	Not measured	5.62	0.04
3	13	0 h post-mortem (n=13)	6.53	0.23	6.53	0.20
		24 h post-mortem (n=9)	5.83	0.08	5.71	0.12
		48 h post-mortem (n=9)	5.65	0.09	5.61	0.09
4 (Friday)	11	0 h post-mortem (n=11)	6.60	0.22	6.65	0.19
		72 h post-mortem (n=11)	Not measured	Not measured	5.71	0.04
5	12	0 h post-mortem (n=12)	6.86	0.26	6.83	0.28
		24 h post-mortem (n=9)	6.05	0.15	5.9	0.10
		48 h post-mortem (n=9)	5.72	0.08	5.64	0.06
TOTAL	61	0 h post-mortem (n=61)	6.58	0.25	6.57	0.29
	25	24 h post-mortem (n= 25)	5.91	0.14	5.78	0.12
	25	48 h post-mortem (n=25)	5.69	0.08	5.64	0.07
	24	72 h post-mortem (n=24)	Not measured	Not measured	5.67	0.06

There was a significant difference (95% confidence level) between the mean pH measured at 0 h post-mortem and 24 h post-mortem and between the meat pH measured at 24 h and 48 h post-mortem (Table 6).

Table 6. Statistical tests utilised to analyse the difference between the meat pH measured at 0 h post-mortem and 24 h post-mortem and between the meat pH measured at 24 h and 48 h post-mortem.

Variable	Statistical test utilised*	Group 1	Group 2	P-value	95% CI for mean difference	Conclusion
Difference between the mean pH at 0 h (group 1) and 24 h post-mortem (group 2)	Two Sample T-test	x = 6.58 (sdev = 0.25)*2	x = 5.91 (sdev = 0.14)*2	0.000	0.564-0.776	As the test statistic value is minor than the critical value, we reject the null hypothesis. Therefore, we conclude that the mean pH at 0 h is **significantly different** than the mean pH at 24 h post-mortem
Difference between the mean pH at 24 h (group 1) and 48 h post-mortem (group 2)	Two Sample T-test	x = 5.91 (sdev = 0.14)*2	x = 5.69 (sdev = 0.08)*2	<0.001	0.155-0.285	As the test statistic value is minor than the critical value, we reject the null hypothesis. Therefore, we conclude that the mean pH at 24 h is **significantly different** than the mean pH at 48 h post-mortem

*Note: The critical value was calculated using a 5% significance level (α).
*2 Note: "x" represents the mean and "sdev" represents the standard deviation.

The results of the meat pH measurement are presented in Figures 9-12. As forementioned, some meat pH measurements were not included in this study since it was conducted under commercial conditions and some of the carcasses (n=12) were sold before the measurements.

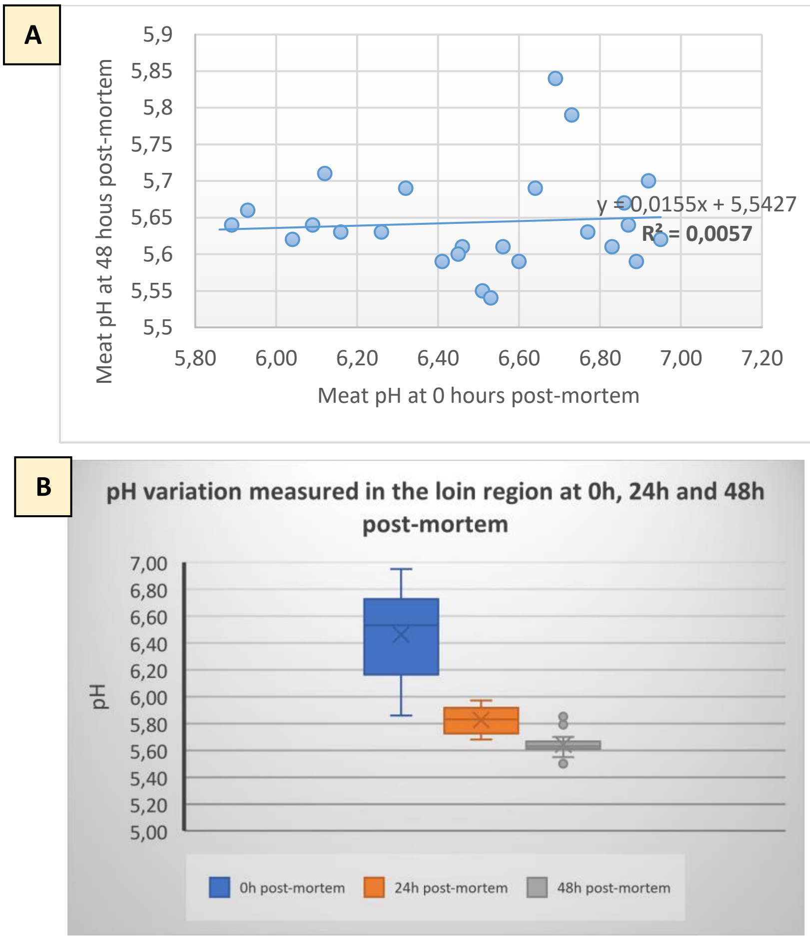
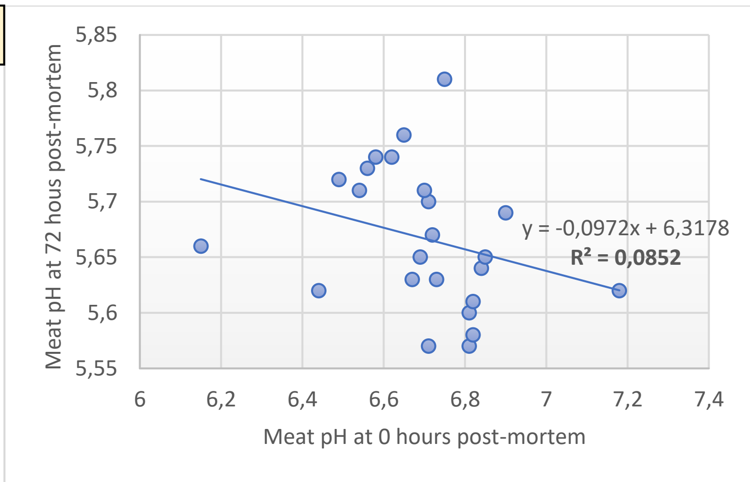


Figure 9. (A) Relation between meat pH measured in loin region at 0 and 48 hours post-mortem of Barrosã carcasses slaughtered on three different days at the PEC Nordeste Slaughterhouse (n=37) and (B) Variation of meat pH measured in the loin region at 0, 24 and 48 hours post-mortem of Barrosã carcasses slaughtered on three different days at the PEC Nordeste Slaughterhouse (n=37).

A



B

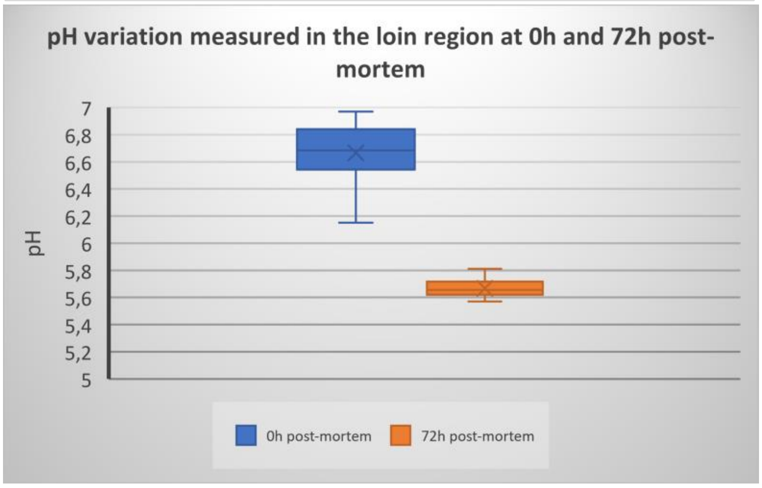
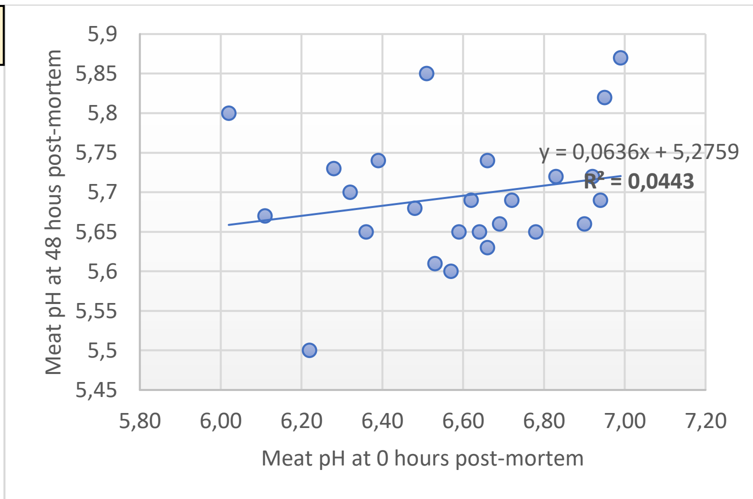


Figure 10. (A) Relation between meat pH measured in loin region at 0 and 72 hours post-mortem of Barrosã carcasses slaughtered on two different days at the PEC Nordeste Slaughterhouse (n=24) and (B) Variation of meat pH measured in the loin region at 0 and 72 hours post-mortem of Barrosã carcasses slaughtered on two different days at the PEC Nordeste Slaughterhouse (n=24).

A



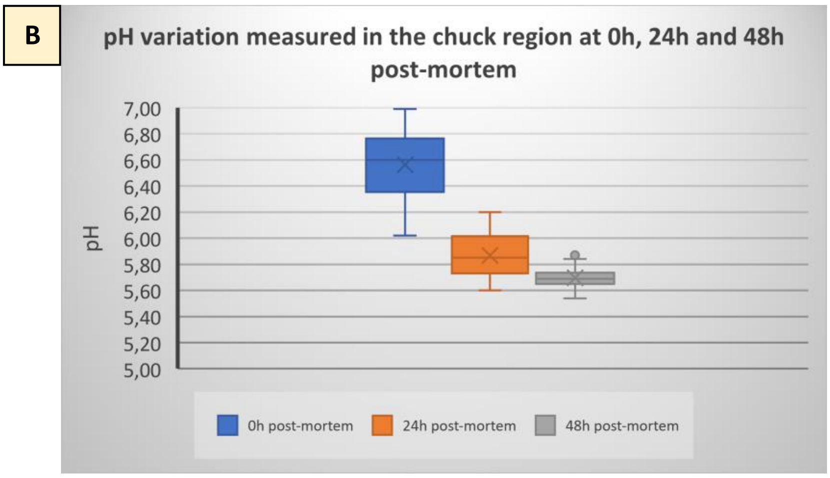


Figure 11. (A) Relation between meat pH measured in chuck region at 0 and 48 hours post-mortem of Barrosã carcasses slaughtered on three different days at the PEC Nordeste Slaughterhouse (n=37) and (B) Variation of meat pH measured in the chuck region at 0, 24 and 48 hours post-mortem of Barrosã carcasses slaughtered on three different days at the PEC Nordeste Slaughterhouse (n=37).

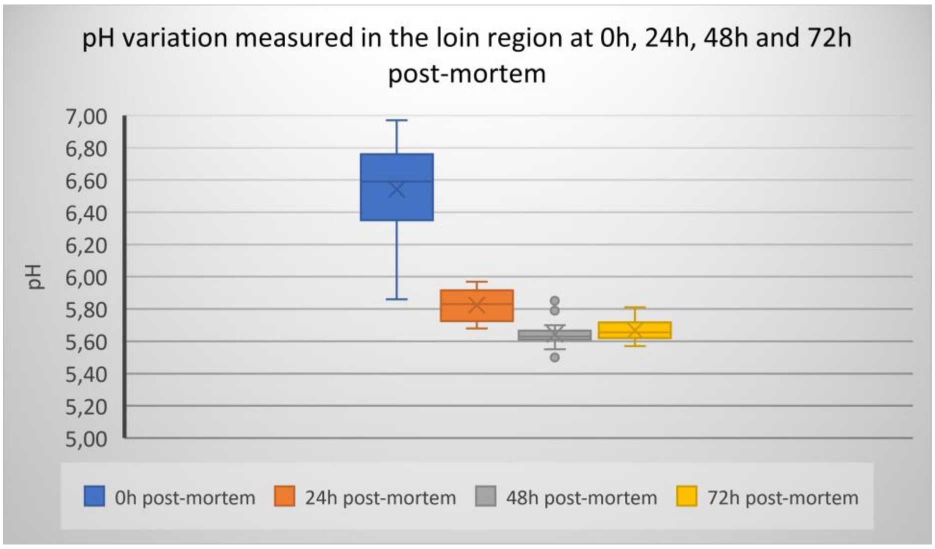


Figure 12. Variation of meat pH measured in the loin region at 0, 24, 48 and 72 hours post-mortem of Barrosã carcasses slaughtered on five different days at the PEC Nordeste Slaughterhouse (n=61).

Results exhibited in Figures 9 to 11 (box whisker plots and scatterplots), suggest that it is not possible to predict the carcasses' pH at 48 or at 72 hours post-mortem based on the pH at 0 hours. The correlations obtained are very low, showing no association between pH at 0 and pH at 48 or 72 h post-mortem, supporting this statement.

Detailed characterization of five abnormal pH carcasses

Among the slaughtered animals, five exhibited a final pH value (at 48 or 72 hours post-mortem) of 5.8 or above, which was the threshold, used in this study, for classifying meat pH as problematic. Notably, three of these slaughters occurred on the same day, while the remaining two occurred on two different days. The five animals were male, with eight months of age. The live weight of the animals exhibited a considerable range, spanning from 160.0 kg to 274.0 kg, with a mean of 211.2 kg (standard deviation = 42.9kg). The hot carcass weights exhibited a range from 77.9 kg to 134.7 kg, with a mean of 107.9 kg (standard deviation = 23.2kg). The results of these carcasses are presented in Table 7. None of the five animals exhibited signs of disease or injury at the lairage, and their behaviour was not different compared to the other animals.

Table 7. Description of the five Barrosã carcasses with problematic pH results (meat pH ≥5.8), measured in chuck (n=4) or loin region (n=1) at 48 or 72 hours post-mortem. And mean age (months), live weight (kg) and hot carcass weight (kg) of the five carcasses. Animals A3, A4 and A5 were slaughtered on the same day, and animals A1 and A2 were slaughtered on two different days.

					pH at 0 h		**pH at 24 h**		**pH at 48 h**		**pH at 72 h**
Animal	**Sex**	**Age (months)**	**Live Weight (kg)**	**Hot Carcass Weight (kg)**	**Chuck**	**Loin**	**Chuck**	**Loin**	**Chuck**	**Loin**	**Loin**
A1	M	8	274	134.7	6.51	6.69	5.88	5.85	5.85	5.84	
A2	M	8	224.5	127.6	6.67	6.75					5.81
A3	M	8	199.5	98.7	6.02	5.89	6.2	5.92	5.8		
A4	M	8	160	77.9	6.95	6.73	6.05	5.9	5.82		
A5	M	8	198	100.5	6.99	6.95	6.11	5.97	5.87		
Mean		8	211.2	107.9							
Standard deviation			42.9	23,2							

The ability of pH at 24 hours to predict pH at 48 or 72 h

A total of 25 carcasses were analysed for meat pH measurements at 24 hours post-mortem in the **chuck** region. Of these, 19 exhibited a pH value ≥ 5.8, while the remaining 6 exhibited a pH value below 5.8. However, at 48 hours post-mortem measurement in the chuck region, only 4 carcasses exhibited a pH value ≥ 5.8. This indicates that the number of false positive (FP) results at 24 hours is 15 and the predictive positive value (PPV) of the pH measurement at 24 hours post-mortem was 21.1%. This means that if the decision to reject the carcasses is based on the meat pH at 24 hours post-mortem, 15 of 19 carcasses are inadequately rejected.

Conversely, of the 25 carcasses analysed for meat pH measurement at 24 hours post-mortem in the **loin** region, 12 exhibited pH ≥ 5.8. However, at 48 hours post-mortem, this number was reduced to one, resulting in 11 FP results and a PPV of 8.3%. This means that if the decision to reject the carcasses is based on the meat pH at 24 hours post-mortem, 11 of 12 carcasses are inadequately rejected.

3.6 Barrosã PDO meat pH history (between May 2020 and May 2024)

The period under analysis, spanning from May 4th 2020 to May 17th 2024, encompassed the slaughter of 4800 animals, of which only 3978 had their meat pH measured. The majority of these 3978 animals were between seven and nine months of age, with age varying between four and eleven months, as illustrated in Figure 13A. The mean live weight at slaughter per age group (i.e. months of age) varied between 188.1 kg (standard deviation = 29.49 kg), for the five months calves, and 208.4 kg (standard deviation = 32.30 kg), for the eight months calves (Figure 13A).

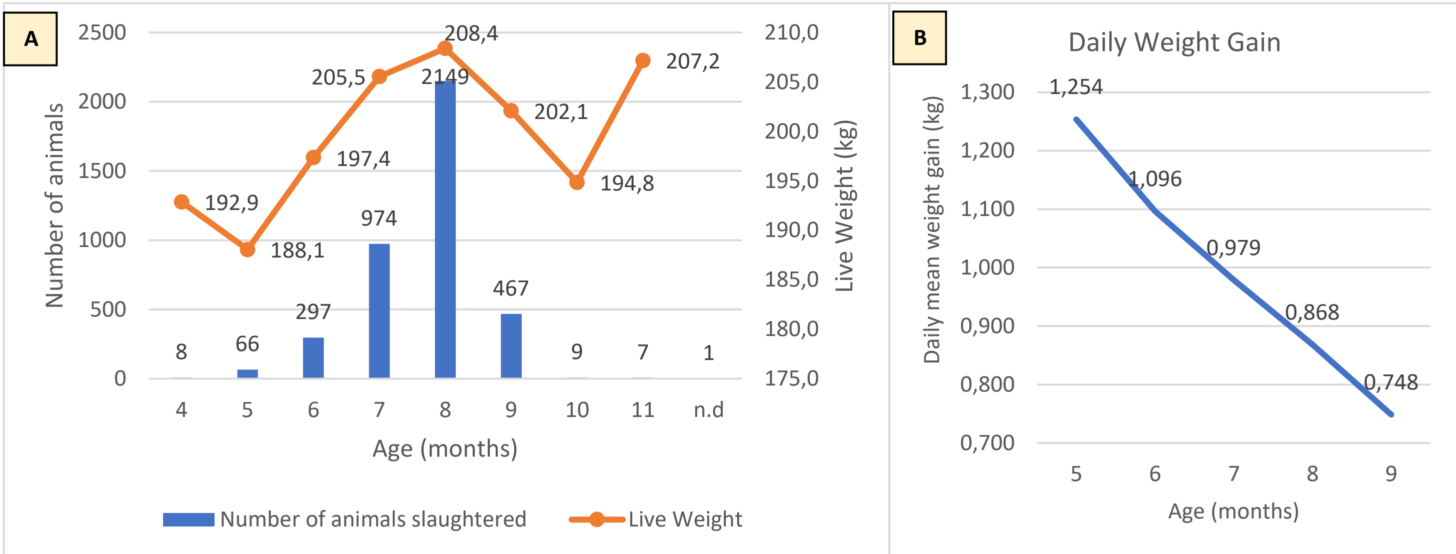


Figure 13. (A) Age (in months) and the mean live weight at slaughter per age group (months of age), of the 3978 Barrosã DOP calves slaughtered in the period from May 4th 2020 to May 17th 2024 at the PEC Nordeste Slaughterhouse. (B) Mean Daily Weight Gain (in kg/day) of the 3953 Barrosã DOP calves slaughtered between the ages of 5 and 9 months at the PEC Nordeste Slaughterhouse. (Source: CAPOLIB's data base)

The mean daily weight gain of the Barrosã DOP animals slaughtered between 5 and 9 months of age was calculated (Figure 13B). There is a decline in the daily weight gain as the age increases, from 1.254 kg/day at 5 months old to 0.748 kg/day at 9 months old. The crude mean daily gains extracted from the database's data appear to be inaccurate and non-realistic representation of the actual data.

The meat pH was measured for the 3978 carcasses by the engineers working at the slaughterhouse and the results were divided into four categories: <5.8; [5.8; 5.9[; [5.9; 6.0[and ≥6.0. Due to the work schedule at the slaughterhouse, 1539 measurements were done at 24 hours post-mortem while the remaining 2439 measurements were done at 72 hours post-mortem. Results are presented in Figure 14.

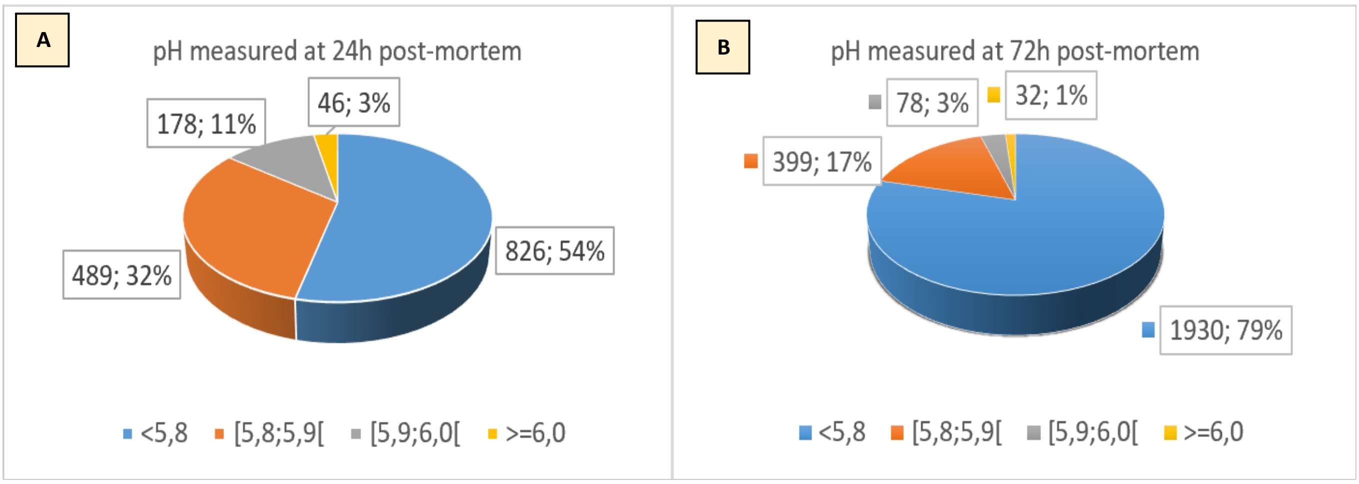


Figure 14. Barrosã PDO meat pH results divided into four categories (<5.8; [5.8; 5.9[; [5.9; 6.0[and ≥6.0) of the total 3978 calves slaughtered in the period from May 4th 2020 to May 17th 2024 at the PEC Nordeste Slaughterhouse. (A) 1539 measurements were done at 24 hours post-mortem while (B) the remaining 2439 measurements were done at 72 hours post-mortem. The pH measurements were done by inserting a portable meat pH and temperature meter in the loin region at a comparable depth and location in each carcass. (Source: CAPOLIB's data base)

The proportion of meat pH non-problematic results (<5.8) measured at 24 hours post-mortem was 54%, while at 72 hours post-mortem was 79%. This proportions' difference ranges between 22.1% and 27.9% (95% CI = 0.2206 – 0.2794) and is statistically significant (confidence level = 95%; p-value = 0.000). The Attributable Risk (AR) represents the portion of meat pH results classified as non-problematic (pH >5.8) due to the pH measurement being conducted at 72 h post-mortem, rather than at 24 h post-mortem. The AR calculated in study was 31% (calculated as the quotient between 25 and 79).

The relation between sex and meat pH value was also assessed. At 72 h post-mortem, the proportion of problematic results (pH ≥5.8) in females was 0.183 while in males was 0.226. There was a significant difference between the proportion in females and males (p-value = 0.0102; confidence level of 95%). At 24 h post-mortem the proportion of problematic results for females and males was respectively 0.392 and 0.502. Once more, there was a significant difference between the proportion in females and males (p-value < 0.0001; confidence level of 95%).

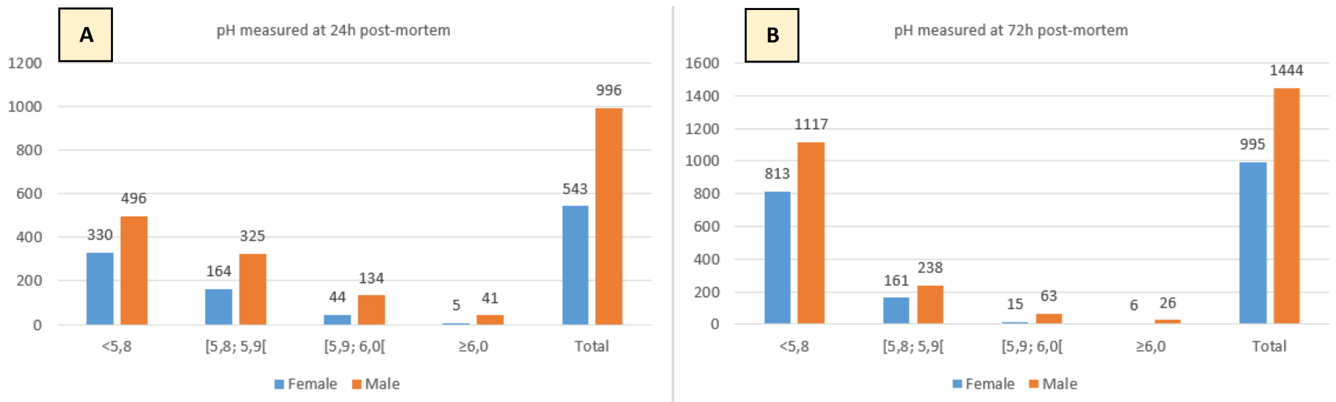


Figure 15. Meat pH categories divided by sex of the total 3978 Barrosã PDO carcasses analysed from the slaughters in the period from May 4th 2020 to May 17th 2024 at the PEC Nordeste Slaughterhouse. (A) 1539 measurements were done at 24 hours post-mortem while (B) the remaining 2439 measurements were done at 72 hours post-mortem. (Source: CAPOLIB's data base)

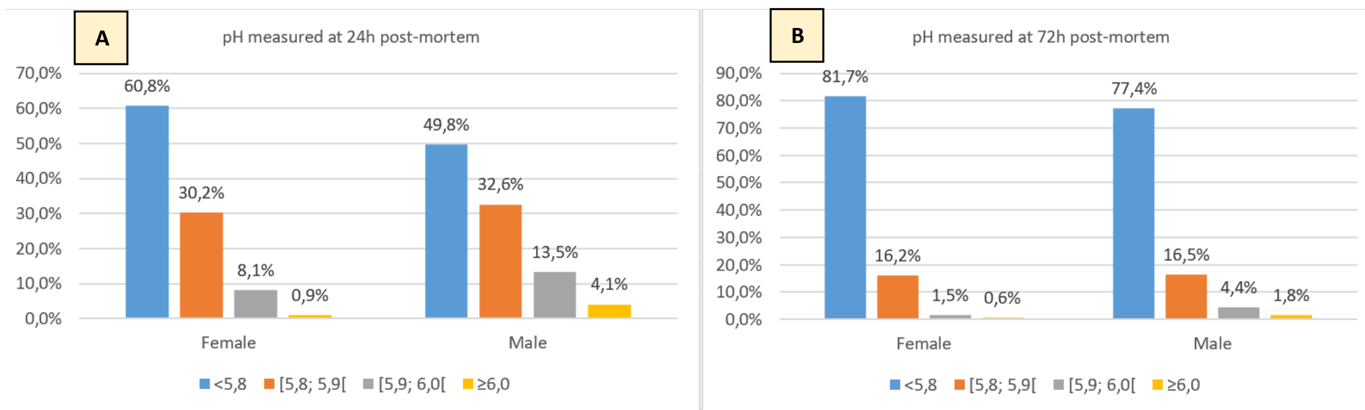


Figure 16. Results of the meat pH measurements divided by sex and pH category of the Barrosã PDO calves slaughtered from May 4th 2020 to May 17th 2024 at the PEC Nordeste Slaughterhouse. (A) 1539 measurements (543 females and 996 males) were done at 24 h post-mortem while (B) 2439 measurements (995 females and 1444 males) were done at 72 h post-mortem. (Source: CAPOLIB's data base)

4. DISCUSSION:

This study represents a novel approach to understanding the production system of the Portuguese cattle breed, the Barrosã. There is a paucity of information on this breed, which is largely due to the fact that Barrosã breeding has been an agricultural activity that has been passed down through generations and based on the experience of the farmers. This study assessed the entire production chain, from the farm to the slaughterhouse, which enables the documentation of the animals' journey. Furthermore, there are no examples of the type of questionnaires used in this study, which makes it a grassroots work.

This study is of significant importance in terms of promoting knowledge about the Barrosã breed and the growth of the breed. It does this by exposing the breed's characteristics and production system.

The characterisation of the Barrosã production system demonstrated that the rearing of these animals can be accomplished in a multitude of ways. The system employs a variety of pasture regimes, with some animals being reared exclusively indoors, others pasturing with their mothers, and others remaining indoors until a specified age before going to pasture or vice versa. The housing system also varies between farms. Some farms house calves collectively in a single pen, while others house calves and their respective mothers in individual pens. Some farms house all animals (calves and mothers) in a single pen. Feeding is somewhat disparate across the various farms, with hay being a consistent element across all farms, but introduced at different ages in different farms. Some farms also employed the use of different supplements, including potatoes, different cereals or grains, concentrates, and salt blocks, in accordance with their beliefs and empiric knowledge. Furthermore, the vaccination routine and sanitary security measures varied between farms. A further distinction between farms is the breeding method employed, with some utilizing natural mating and others resorting to artificial insemination or a combination of both.

The exceptional rusticity characteristic of the Barrosã breed (FAO, 2018), which is evidenced by the low morbidity and mortality rates, explains the low demand for veterinary services in this production system. However, it may also indicate that some of the diseases are underdiagnosed and that many of the causes of death remain unexplained. This is evidenced by the findings of this study, which identified eleven deaths, six (55%) of which were attributed to causes that could not be determined, and three (27%) of which were the result of accidental causes.

The focus of this study was not to extensively characterise the Barrosã breed. Instead, it was to paint a simple picture of the breed and its unique production system. More information on it can be found in the work of AMIBA, 1997, 2001, 2023; Dias & Maia, 2020 and FAO, 2018.

The Barroso and Minho regions are known for their rough country roads with many stops and turns, and, sometimes, bad pavement. This makes the journey to the slaughterhouse more challenging and eventually more stressful for the animals, possibly increasing the incidence of dark-cutting meat (Buckham-Sporer et al., 2023). Accompanying and characterising the transportation, made it possible to corroborate the rough road condition, nevertheless, it was not possible to identify it as a cause for the elevated pH problems. Furthermore, the animals' transport to the slaughterhouse varies greatly with the origin farm and its location. The manner in which farmers load animals onto the truck varies considerably. Some use shouting, sticks, or aggressive movements, while others use calm voices, and patient and calm methods to direct the animals, and prepare a distraction-free race for them to follow to the truck ramp. Additionally, animals react differently to the loading procedure. Some become distressed, nervous, aggressive or even frightened, while others approach the truck calmly and without hesitation. The number of animals loaded at each farm varies considerably, with some farms loading the majority of the animals transported and others loading only one or two animals. This can also impact animals' distress, as cattle are highly social animals and live in established groups and hierarchies, any change to their group can lead to social stress (Nielsen et al., 2020).

The environmental temperature plays a crucial role in animals' comfort during transportation and it is of great importance to respect animals' thermoneutral zone (from 15 °C to 25 °C for young calves) below and above which animals experience cold and heat stress, impacting their physiological and metabolic activities (Buckham-Sporer et al., 2023). Regardless of not being able to determine the vehicles' temperature during transport, none of the animals demonstrated signs of cold or heat stress upon arrival at the slaughterhouse. During the summer peak (months of July, August and September) the situation may change as the temperature rises considerably in this region, and measures must be taken to ensure animals' comfort during transport.

The slaughterhouse work was smooth and organised, the facilities were reasonably well designed and the personnel was instructed regarding animals' welfare. Animals did not show signs of stress in the lairage and were conducted calmly through the race leading to the stunning point. The connection between the stunning and the bleeding point could be improved as the time between these phases took between 60 and 130 seconds, with an average time of 87 seconds. The use of a penetrating captive bolt device should be followed by the sticking procedure as soon as possible, ideally within 60 seconds, otherwise, animals regain consciousness (FAO, 2004).

It should be noted that this study was not without limitations. Firstly, the study was conducted in a commercial setting, which precluded the possibility of measuring the meat pH of all the carcasses at the stage accompanying the slaughterhouse work. Similarly, the data from the previous four years' slaughtering operations was not accessible in its entirety. As a result, the sample size was somewhat reduced, which in turn made it more challenging to achieve a statistically significant sample size. The reduced sample size also precluded the calculation of a predictive equation for the meat pH value at 48 hours post-mortem based on the pH value immediately after slaughter, at 0 hours post-mortem.

Secondly, the questionnaires employed a qualitative and descriptive approach, and the responses to the questionnaire regarding the farms and the production systems were largely dependent on the farmers' sincerity and honesty.

The average daily weight gain of the animals slaughtered was calculated, but the reliability of the animals' ages recorded on CAPOLIB's database was questionable. As illustrated in Figure 13A, the mean live weight of the animals slaughtered at nine and ten months of age, between May 4th 2020 and May 17th 2024, is lower than the mean live weight of the animals slaughtered at eight months of age. This raises questions about the veracity of the age determination. This unreliability may result from the economic valorisation of calves' carcasses to the detriment of adult animals' carcasses.

Furthermore, the selection of the twenty-two Barrosã farms sample was based on the problem under study (the elevated meat pH for the previous four years), combined with the subjective experts' assessment. This makes the external validity of the sampling procedure somewhat questionable. The two highest proportion of problematic results values corresponded to two control farms, three control farms had a proportion value higher than the mean value, and only one farm from the problem group had a proportion value below the mean value. In addition, no statistically significant difference was observed between the proportion of problematic pH results in the control and problem groups (p-value = 0.478). Consequently, following the farms' evaluation, it was concluded that the two groups (control and problem) exhibited no discernible difference in relation to the proportion of problematic results. However, there was a significant difference in farm size between the groups (p-value = 0.005), which could explain the different management strategies employed.

To add to the limitations of this study, some of the meat pH measurements are not comparable, due to the discrepancy in the timing of the measurements. The meat pH value at 24 h post-mortem is significantly different from the meat pH value at 48 h post-mortem (p-value <0.001), with indicates that measurements taken at 24 h should not be compared to measurements taken at 48 h post-mortem. The decline in meat pH is not complete at 24 hours post-mortem, as illustrated in Figures 9-12, which suggests that the meat pH measurement at 24 hours post-mortem is not an accurate indicator of meat quality. Furthermore, as aforementioned the positive predictive value (PPV) of the measurement at 24 hours was 21.1% for the chuck region and 8.3% for the loin region. This indicates that 78.9% of the carcasses classified as problematic (pH ≥ 5.8) by measuring the pH on the chuck region were not considered problematic at 48 hours post-mortem. Conversely, 91.7% of the carcasses classified as problematic (pH ≥ 5.8) by measuring the pH on the loin region were not considered problematic at 48 hours post-mortem. It is evident that deciding on whether to reject certain carcasses based on the 24 hours post-mortem results is not a reasonable strategy, especially when the chuck region is used to the measurement. It is therefore recommended that meat pH measurement should be conducted at 48 hours post-mortem, when the pH decline has reached a relatively stable point.

The Attributable Risk (AR) calculated in this study, represents the portion of meat pH results classified as non-problematic (pH >5.8) due to the pH measurement being conducted at 72 h post-mortem, rather than at 24 h post-mortem. Therefore, in this case, the AR is 31% (calculated as the quotient between 25% and 79%). This signifies that 31% of the non-problematic carcasses at 72 h post-mortem would be classified as problematic at 24 h post-mortem, and in some extreme cases (pH ≥6.0) rejected.

The meat quality, specifically its pH level, is dependent on a number of factors, as previously discussed. It is therefore challenging to ascertain the underlying cause of the recent meat pH issues observed in Barrosã PDO meat. These issues may be attributed to a multitude of factors, encompassing the farm and production system, transportation, and the slaughterhouse environment and work practices. The underlying causes of the elevated meat pH observed in Barrosã meat in the previous years remain unclear, despite the evaluation of several potential variables in the present study. It is therefore recommended that future studies consider additional variables to gain a deeper understanding of the factors associated with this phenomenon. Aside from the farm size, the two groups were not different, suggesting that the elevated pH problem may be explained by individual factors, including animals' genetics, which could influence animals' response to the stress stimuli and the biochemical impact of that stress on the animal. Additionally, slaughter or transport problems on a particular day may also explain pH problems in some carcasses, as it is not dependent on the origin farm and its management.

The different proportion of problematic meat pH between females and males at 24 h and 72 h post-mortem found in this study (p-value < 0.0001 and p-value = 0.0102, respectively) was somewhat unexpected. That relation should be studied profoundly, because it may have economic implications to the farmers and farmers' associations.

Although the meat pH decline and its underlying mechanisms have been previously studied and described, there is a need to investigate the impact of the diverse factors related to the production system and transportation on the pH evolution along time. Additionally, the threshold for classifying meat pH as problematic remains unclear in the existing literature. It would be beneficial to study the pH value above which meat quality is compromised. Furthermore, it would be advantageous to investigate the temporal evolution of meat pH, particularly in the Barrosã breed, with the aim of developing an equation to predict the final pH value based on the pH value measured immediately post-slaughter.

The meat pH evolution observed in this study does not follow the standard decline presented in other works (Matarneh et al., 2017) for the Charolais breed. Matarneh et al. (2017) found that at 24 h post-mortem all the carcasses evaluated (n=3) had pH values below 5.8. These findings differ from the results of this study on the Barrosã breed, which demonstrated that at 24 h post-mortem the mean pH value of the carcasses evaluated (n=25) was 5.91 (standard deviation = 0.14), as represented in Table 5. This discrepancy may be attributed to a distinct pH decline pattern in the Barrosã breed, extending the decline after 24 h post-mortem. In contrast, as illustrated in Figure 12, the pH at 48 h post-mortem did not differ from the value at 72 h post-mortem. This may indicate that the pH decline is finished at 48 h. The explanation for the later pH decline can be attributed to various factors, such as Barrosã breed physiological differences, different nutrition strategies, and different chilling and maturation conditions at the slaughterhouse, among others.

New strategies are emerging to address animal welfare and meat quality. These strategies aim to reduce the stress experienced by animals during transportation and slaughter and regulate animals' stress response. One such strategy is the administration of maternal appeasing substance (mBAS) before slaughter (Mackey et al., 2024) in order to improve carcass dressing by diminishing the stress resulting from the slaughter process. This approach may help to mitigate the impact of the stress on meat quality. Further research on these topics is valuable.

Finally, there is a paucity of information on the Barrosã breed, which presents an opportunity for further research. This could potentially stimulate interest in the breeding and commercialisation of these animals.

5. CONCLUSIONS:

The study demonstrated that the Barrosã production system is a highly intricate and multifaceted one, encompassing a multitude of distinct methodologies for rearing animals.

Although not able to find the factors that explain the elevation in the pH of the Barrosã PDO meat, this study made significant improvement in the realisation that numerous variables need to be considered when studying this topic, including the farms' conditions, as well as the transportation and slaughter processes. Furthermore, it is greatly important to establish different criteria for the categorisation of meat quality, from the ones currently used. As demonstrated in this study, the pH at 24 hours post-mortem is not the optimal indicator for this purpose, because the pH decline is not finished at that point, and there is a significant probability of error associated with that measurement. Additionally, it is of the utmost importance that the data available on the Barrosã animals, namely the animals' age, be meticulously determined in order to ensure reliability on animals' age at slaughter and allow the calculation of distinct parameters that depend on the age, such as the mean daily weight gain.

In conclusion, numerous variables can be enhanced in the Barrosã PDO meat production system with the objective of foresting the growth of this cattle breed and promoting its potential in the contemporary meat industry. However, these changes may require time for farmers to adjust and to perceive the impact. Barrosã farmers exhibit a profound pride in their animals, demonstrating a keen interest not only in the expansion of the industry but also in the advancement of the production system and the enhancement of animal welfare and meat quality.

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ANNEXE A

AMIBA's database analysis

Table A1. Description of the number of farms and the number of adult animals (males and females) of the Barrosã breed per municipality, along with the calculated mean and standard deviation of the number of adult animals per farm (Farm size) divided by municipality and district.

District	Municipality	Number of farms	Number of Males	Number of Females	Total of animals	Number of animals per farm (Farm size)	
						Mean	Standard deviation
Braga	Amares	10	2	39	41	4,10	6,55
	Barcelos	5	2	8	10	2,00	1,79
	Cabeceiras de Basto	141	36	670	706	5,35	6,40
	Celorico de Basto	4	0	15	15	3,75	2,05
	Fafe	12	11	28	39	3,25	4,19
	Póvoa de Lanhoso	16	5	21	26	2,00	2,86
	Terras de Bouro	117	16	358	374	3,63	4,53
	Vieira do Minho	167	74	979	1053	7,16	8,38
	Vila do Conde	1	1	2	3	3,00	0,00
	Vila Nova de Cerveira	4	2	15	17	4,25	4,09
	Vila Nova de Famalicão	5	0	8	8	2,00	2,92
	Vila Real	1	0	18	18	18,00	0,00
	Vila Verde	76	15	249	264	3,67	4,86
	Vizela	2	0	2	2	1,00	1,00
Castelo Branco	Castelo Branco	4	0	18	18	4,50	2,69
Coimbra	Miranda do Corvo	1	0	1	1	1,00	0,00
Évora	Alandroal	1	0	39	39	39,00	0,00
	Estremoz	1	1	12	13	13,00	0,00
	Montemor-o-Novo	10	3	82	85	17,00	18,25
	Mora	2	0	1	1	1,00	0,00
Funchal	Funchal	2	1	4	5	5,00	0,00
Guarda	Almeida	1	1	17	18	18,00	0,00
	Gouveia	1	1	7	8	8,00	0,00
Guimarães	Guimarães	19	2	27	29	1,93	3,66
Leiria	Alcobaça	1	0	2	2	2,00	0,00
	Porto de Mós	3	0	6	6	3,00	0,00
Porto	Matosinhos	1	1	1	2	2,00	0,00
	Penafiel	1	0	2	2	2,00	0,00
	Porto	1	0	1	1	1,00	0,00
	Santo Tirso	9	5	22	27	3,38	3,08
Santarém	Chamusca	5	0	1	1	1,00	0,00
	Ourém	1	0	1	1	1,00	0,00
	Santarém	1	0	2	2	2,00	0,00
Viana do Castelo	Arcos de Valdevez	251	57	1225	1282	5,43	7,94
	Arruda dos Vinhos	1	0	0	0	0,00	0,00
	Caminha	1	0	2	2	2,00	0,00

	Melgaço	30	17	377	394	17,91	24,40
	Monção	34	10	301	311	9,42	18,65
	Paredes de Coura	26	7	171	178	7,74	15,89
	Ponte da Barca	106	10	309	319	3,04	4,19
	Ponte de Lima	21	5	84	89	5,93	9,68
	Valença	3	0	19	19	6,33	5,56
	Viana do Castelo	8	0	19	19	2,71	3,45
Vila Real	Boticas	73	18	428	446	6,66	5,66
	Braga	4	2	45	47	11,75	11,26
	Chaves	2	1	25	26	13,00	6,00
	Montalegre	276	115	2181	2296	9,04	10,93
	Ribeira de Pena	3	0	18	18	6,00	3,56
Total		1465	421	7862	8283	6,21	9,32

Note: This table encompasses data of the Barrosã breed population, which includes not only Barrosã PDO animals and farms but also non-PDO Barrosã animals and farms.

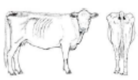
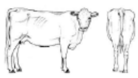
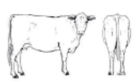
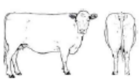
ANNEXE B

AMIBA's morphologic classification tables for the Barrosã breed

Table B1. Associação de Criadores da Raça Barrosã, AMIBA's behabiour classification table for the Barrosã breed. Source: Manual de Classificação Morfológica Linear – Raça Barrosã.

	Pontuação	Comportamento
	1	O animal move-se sem medo em direção ao classificador e aproxima-se do contacto.
	2	O animal não se move, é indiferente ao trabalho do classificador.
	3	O animal move-se sem se preocupar.
	4	O animal move-se rapidamente, está preocupado e se as mães estiverem próximas, ele terá tendência a refugiar-se atrás delas.
	5	O animal foge porque tenta afastar-se do classificador o mais rápido possível.
	6	O animal mostra sinais de grande nervosismo sem atacar (movimentos da cabeça, olhar fixo no classificador).
	7	O animal investe para o classificador.

Table B2. Associação de Criadores da Raça Barrosã, AMIBA's body condition scoring table for the Barrosã breed. Source: Manual de Classificação Morfológica Linear – Raça Barrosã.

	Pontuação	Condição	Descrição
	1	Muito Magra	Extremamente raquítica, próxima da morte por inanição. Costela, espinha dorsal e anca muito proeminentes. Nenhum tecido gorduroso visual.
	2	Magra	Um pouco definhada. Costelas, espinha dorsal e anca proeminentes
	3		Costelas visualizadas individualmente, mas não tão salientes. Um pouco de carne ao longo da espinha dorsal.
	4	Intermédia	Costelas individuais pouco ou não evidentes. Pouca gordura sobre costelas e ossos da anca. Pode apalpar espinha, não pontiaguda.
	5	Ótima	Moderada ou boa. Gordura palpável sobre as costelas e qualquer lugar de garupa. Espinha dorsal pouco visível.
	6		Necessita de pressão para apalpar espinhas. Considerável gordura palpável sobre as costelas.
	7	Gorda	Gorda. Um pouco de gordura no peito, boa quantidade de gordura sobre as costelas. Acúmulo de gordura na região da garupa.
	8		Muito gorda. Peito repleto e grande depósito de gordura sobre as costelas, garupa, inserção da camada e vulva.
	9	Muito Gorda	Extremamente gorda. Estruturas ósseas não visíveis e não palpáveis.

ANNEXE C

Illustrative photography of the Slaughterhouse's equipment

Figure C1. Rubber structures on the sides of the race leading animals to the stunning point, at the PEC Nordeste Slaughterhouse.

ANNEXE D

Illustrative photography of the Barrosã Production System

Figure D1. Barrosã calves of one of the farms visited and evaluated.

Figure D2. Barrosã calf (in front) and bull (on the back) of one of the farms visited and evaluated.

Assessment of the production system, transportation, and slaughter in a sample of Barrosã PDO herds

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