UNIVERSIDADE FEDERAL DO PARANÁ

MATHEUS DENIZ

THERMAL ENVIRONMENT AND SOCIAL HIERARCHY INFLUENCE THE LOCATION AND BEHAVIOR OF DAIRY COWS IN A SILVOPASTORAL SYSTEM

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THERMAL ENVIRONMENT AND SOCIAL HIERARCHY INFLUENCE THE LOCATION AND BEHAVIOR OF DAIRY COWS IN A SILVOPASTORAL SYSTEM

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Coorientador: Prof. Dr. Marcos Martines do Vale

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Presidente da Banca Examinadora

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04/03/2022 14:32:42.0
FREDERICO MÁRCIO CÔRREA VIEIRA
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Assinatura Eletrônica
04/03/2022 15:14:28.0
MAITY ZOPOLLATTO
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

Assinatura Eletrônica
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PUBLICATIONS

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“A compaixão para com os animais é das mais nobres virtudes da natureza humana.”
Charles Darwin

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RESUMO

O conhecimento das estratégias comportamentais de vacas leiteiras dentro de um sistema silvipastoril pode nos ajudar compreender a termodinâmica das vacas. Portanto, neste estudo, avaliamos a influência do microclima, indicadores de conforto térmico e hierarquia social nos comportamentos diurnos e localização (sombra ou sol) de vacas leiteiras criadas em um sistema silvipastoril de clima subtropical. Para alcançar esse objetivo, a tese foi dividida em V capítulos. No capítulo I, realizamos uma revisão sistemática da literatura científica sobre os efeitos do sistema silvipastoril no ambiente físico, indicadores de conforto térmico, comportamento e respostas fisiológicas de bovinos leiteiros. Nossa revisão destacou que os comportamentos (por exemplo: ócio e ruminação) associados a postura deitado e o comportamento social e têm sido pouco explorados nos estudos. Assim, na tentativa de preencher a lacuna do comportamento social, no capítulo II avaliamos quais características fenotípicas de vacas leiterias determinam sua posição social no contexto de rebanho misto (vacas com e sem chifres) e a influência da posição social no tempo em que as vacas permanecem no cocho de alimentação. Para isso, foram calculados os valores de dominância para cada animal e o rebanho foi dividido em três categorias sociais: dominante (D), intermediário (I) e subordinado (S). Encontramos que posição social das vacas foi influenciada pela idade, massa corporal e comprimento do corpo; além da posição social influencia o tempo que cada categoria permaneceu no cocho de alimentação. Para que pudéssemos avaliar o microclima e indicadores de conforto térmico do sistema silvipastoril, no capítulo III desenvolvemos e validamos um registrador de dados autônomo (denominado ADEF) para medir variáveis ambientais. O desempenho do ADEF foi satisfatório, demonstrando que é válido como uma ferramenta de baixo custo para medir a variabilidade microclimática na área de interesse. Para que seja possível avançar no conhecimento da termodinâmica das vacas, é necessário transformar dados coletados em informações úteis; assim, no capítulo IV, aplicamos a técnica de mineração de dados para classificar fatores ambientais com potencial de motivar vacas leiteiras a acessarem sombra natural. Através da mineração de dados, encontramos que a radiação solar foi o fator ambiental com maior potencial para classificar a decisão da vaca leiteira de acessar áreas sombreadas. Pelo padrão encontrado em nosso estudo, sugerimos que trabalhos futuros utilizem indicadores de conforto térmico que considerem a radiação solar (ex. Índice de Globo Negro e Umidade – ITGU) para avaliar o conforto térmico de vacas leiteiras criadas em áreas de pastagem. Por fim, associando os conhecimentos do comportamento social (capítulo II) e conforto térmico (capítulos III e IV), no capítulo V, avaliamos a relação entre conforto térmico, hierarquia social, localização das vacas (sombra ou sol) e seus comportamentos diurnos em um sistema silvipastoril. A localização das vacas foi influenciada pelo ITGU e hierarquia social; além desses fatores, o comportamento de deitar foi influenciado pela temperatura superficial do solo. Vacas dominantes foram mais propensas a utilizar as áreas sombreadas para ócio e ruminação deitadas do que vacas subordinadas e intermediárias; ou seja, vacas dominantes eram mais propensas a expressar seus comportamentos de conforto em áreas sombreadas. Em conclusão, através da interdisciplinaridade deste estudo foi possível avançar no conhecimento da termodinâmica de vacas leiteiras criadas em sistema silvipastoril; o qual foi possível através da integração do conhecimento do comportamento diurno e social das vacas, ferramenta de mineração de dados e o uso de sensores precisos e de baixo custo.

Palavras-chave: Abatimento de calor. Bem-estar animal. Biometeorologia. Etologia aplicada. Hierarquia social. Microcontrolador. Mineração de dados. Padrão comportamental. Probabilidade. Áreas sombreadas. Valor de dominância. Zootecnia de precisão.

ABSTRACT

The knowledge of the behavioral strategies of dairy cows within a silvopastoral system (SPS) can help us to understand the cows' thermodynamics. Therefore, in this study, we evaluate the influence of microclimate, thermal comfort indicators, and social hierarchy, on cows' location (shade or sun) and their diurnal behaviors in a silvopastoral system of a subtropical climate. To achieve this aim, the thesis was divided into V chapters. In chapter I, we carried out a systematic review of the scientific literature of the effects of silvopastoral systems on the physical environment, thermal comfort indicators, behavior, and physiological responses of dairy cattle. Our review highlighted that the behaviors (e.g., idle, and rumination) associated with lying down posture and social behavior has been low explored in the studies. Thus, to fill the gap of social behavior, in chapter II, we evaluated which animals' phenotypic characteristics determine the social position in the context of a mixed herd (horned and non-horned cows) and determine the influence of cows' social position on time spent at the feeder. For this, dominance values were calculated for each animal and the herd was divided into three social categories: dominant (D), intermediate (I), and subordinate (S). We found that cows' social position was influenced by age, body mass, and body length; further, the social position influenced the time that each category remained at the feeder. So that we could evaluate the microclimate and thermal comfort indicators of the SPS, in chapter III, we developed and validated an autonomous data logger (named ADEF) to measure environmental variables. The performance of ADEF was satisfactory, demonstrating that it is valid as a low-cost tool to measure microclimatic variability in the area of interest. To advance in the knowledge of the thermodynamics of cows, it is necessary to transform measured data into useful information; so, in chapter IV, we applied the data mining technique to classify environmental factors with the potential to motivate dairy cows to access natural shade. Through data mining, we found that solar radiation was the environmental factor with the greatest potential to classify the dairy cow's decision to access shaded areas. Based on the pattern found in our study, we suggest that future studies use thermal comfort indicators that consider solar radiation (e.g., Black-globe humidity index - BGHI) to assess the thermal comfort of dairy cows raised on pasture areas. Finally, associating the knowledge of social behavior (chapter II) and thermal comfort (chapters III and IV), in chapter V, we evaluated the relationship between thermal comfort indicators, social hierarchy, location of cows (shade or sun) and their diurnal behavior in a silvopastoral system. The cows' location was influenced by BGHI and social hierarchy; further to these factors, the lying behaviors was influenced by the soil surface temperature. Dominant cows were more likely to use shaded areas for idling and rumination lying down than subordinate and intermediate cows; i.e., dominant cows were more likely to performed comfort behaviors in shaded areas. In conclusion, through the interdisciplinarity of this study, it was possible to advance on the knowledge of the thermodynamics of dairy cows raised in a silvopastoral system; which was made possible through the integration of knowledge of the diurnal and social behavior of cows, data mining tool and the use of accurate and low-cost sensors.

Keywords: Animal welfare. Applied ethology. Behavioral pattern. Biometeorology. Data mining. Dominance value. Heat abatement. Microcontroller. Precision livestock farming. Probability. Shaded areas. Social hierarchy.

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LIST OF ABREVIATIONS AND ACRONYMS

SPS	Silvopastoral system
IPCC	Intergovernmental Panel on Climate Change
PR	Paraná
SIMEPAR	Sistema de Tecnologia e Monitoramento Ambiental do Paraná
INMET	National Institute of Meteorology
ISO	International Organization for Standardization
US	United State
PICO	Population Intervention Comparation and Outcome
PRISMA	Preferred Reporting Items for Systematic Review and Meta-analysis Protocols
ADEF	Autonomous data logger to measure environmental variables
IDE	Integrated development environment
PD	Predominant direction
SD	Standard deviation
n	Sample number
GLM	Generalized linear models
R^2	Coefficient of determination
CI	Confidence interval
IRR	Incidence rate ratio
OR	Odds ratio
RSU	Relative standard uncertainty
Freq.	Frequency
Cfb	Humid maritime temperate climate
Cfa	Subtropical humid mesothermic climate
DM	Dry matter
spp.	Species
CP	Crude protein
NDF	Neutral detergent fiber
ADF	Acid detergent fiber
RM	Residue mineral
D	Dominant

I	Intermediate
S	Subordinate
DV	Dominance value
SH	Social hierarchy
SC	Social category
SR	Solar radiation
AT	Air temperature
RH	Relative humidity
ST	Surface temperature
WS	Wind speed
SST	Soil surface temperature
BGT	Black globe temperature
THI	Temperature humidity index
BGHI	Black-globe humidity index
RHL	Radiant heat load
ms	Millisecond
s	Seconds
Min.	Minutes
h	Hour
d	Days
a.m.	Ante meridiem
p.m.	Post meridiem
°C	Degrees centigrade
kg	Kilograms
mm	Millimeters
cm	Centimeters
m	Meters
m^2	Square meters
ha	Hectare
L	Liters
V	Volts
mAh	Milliampere
W	Watts

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1 GENERAL INTRODUCTION

The pasture access for dairy cows is supported by the general public (CARDOSO; VON KEYSERLINGK; HÖTZEL, 2019; SMID et al., 2022) and the scientific literature available shows that dairy cattle are highly motivated to access pasture (ARNOTT; FERRIS; O'CONNELL, 2017; CHARLTON et al., 2013). Adopting pasture-system for livestock requires animals to display complex behaviors, such as grazing and seek shelter (VILLALBA; MANTECA, 2019). Thus, enhancing the diversity of natural pasture systems, through the adoption of rotational grazing systems with shaded provision (MACHADO FILHO et al., 2021), represent management approaches that can be used to improve welfare and prepare the animal for an adaptation to environmental challenges (VILLALBA; MANTECA, 2019). However, knowledge of the thermal environment to which the animals are exposed is a determining factor in farm productivity (RENAUDEAU et al., 2012) and in the animals' quality of life (SHOCK et al., 2016). The technological advances, associated with the low cost of electronic components, have resulted in the emergence of integrated physical devices (NEETHIRAJAN et al., 2017). A set of multiple sensors in a single equipment allows robust and practical measurement of environmental variables for application in livestock farming; thus, the problem can be identified on the farm before it leads to an animal stress condition. Therefore, production systems that improve quality of life for farm animals are gaining attention in the scientific community (see reviews: DAS et al., 2016; HERBUT et al., 2019; KADZERE et al., 2002; POLSKY; VON KEYSERLINGK, 2017), since good health and animal welfare are considered essential to avoid environmental impacts in the livestock sector (BROOM, 2017).

New approaches to data analysis (e.g., data mining) are helping researchers to interpret large databases and improve livestock farming on silvopastoral systems (VOLPI et al., 2021). Silvopastoral systems (SPS) have been highlighted as an important tool to mitigate the effects of environmental factors on grazing animals (DENIZ et al., 2019; KARVATTE et al., 2021). Thus, adopting SPS to provide shade for animals' heat abatement may be key to pasture-based dairy systems; since dairy cows are very motivated to use shaded areas (CARDOSO, et al., 2021). Therefore, understanding the benefits of SPS can influence the adoption of this system; since the use of mechanisms that mitigate environmental stressors in animal production may also address ethical concerns of consumers (CARDOSO, et al., 2018).

The interest to understand and popularize the silvopastoral systems has been motivating research around the world. However, some research that evaluated the thermal comfort indicators of silvopastoral systems did not consider the animals in the studies (DENIZ et al., 2019; KARVATTE et al., 2016; PEZZOPANE et al., 2019); i.e., determined the animals' thermal comfort only with environmental measurements. To know the behavioral strategies of dairy cows within an SPS can help us understand the cows' thermodynamics in these systems. Since the thermodynamics of dairy cows is benefited from thermal comfort improvement and the possibility of choosing between remaining in shaded areas during the hottest hours (DENIZ et al., 2020), also use sunny areas when motivated to do so (DE SOUSA et al., 2021b). Nevertheless, the cows' decision to remain in a certain area can be influenced by the environmental challenge and their social position within the herd. The social position of cows is established through the dominance relationships among animals within the herd (KONDO AND HURNIK, 1990) and can be influenced by some characteristics, such as social learning, age, weight, and horns (BOUISSOU, 1972; ŠÁROVÁ et al., 2013). However, research that included animals in the studies did not consider herd effects (DE SOUSA et al., 2021b; SKONIESKI et al., 2021; VIEIRA et al., 2020) and studies that explicitly deal with social behavior in horned herds compared to non-horned herds are scarce.

To our knowledge, no studies assessed the relationship among thermal environment, social hierarchy, and cows' location in a silvopastoral system. Studies with social hierarchy and cows' behavior have been developed with animals raised in zero-grazing systems (MCDONALD; VON KEYSERLINGK; WEARY, 2020), but when developed in pasture areas it did not consider the thermal environment (BICA et al., 2019, 2020; DE SOUSA et al., 2021a). Thus, questions are raised about the relationship between thermal comfort and social behavior, and the influence of these factors on strategies that dairy herds adopt to use available resources at the production system.

1.1 HYPOTHESES

- Horn is an important feature to establish dominance relationship among dairy cows.

- Low-cost sensors provide reliable results and are efficient in measuring environmental variables with low uncertainty associated of measurements.
- Data mining have the potential to characterize the influence of the thermal environment in the cows' decision to use shaded areas at the pasture.
- Shaded areas of the silvopastoral system provide more comfortable thermal environment for dairy cows to perform their diurnal behaviors.
- Cows' location at the silvopastoral system is influence by thermal environment and social hierarchy.
- Social hierarchy affects the cows' strategies to cope with environmental challenges.

1.2 OBJECTIVES

1.2.1 General objective

To evaluate the influence of microclimate, thermal comfort indicators, and social hierarchy on the diurnal behaviors of dairy cows raised in a silvopastoral system of a subtropical climate.

1.2.2 Specific objectives

- To provide a critical and systematic evaluation of the scientific literature of the effects of silvopastoral systems on the physical environment, thermal comfort indicators, behavior, and physiological responses of dairy cattle.
- To evaluate which animal's phenotypic characteristics are important to determine the social position and determine the influence of social position on the time spent by dairy cows at the feeder.
- To apply the data mining technique to classify which environmental factors have the potential to motivate dairy cows to access shaded areas in a silvopastoral system.
- To develop an autonomous data logger with low-cost sensors to evaluate the thermal environment promoted by the silvopastoral system in different seasons.

- To evaluate the relationship between thermal comfort indicators and social hierarchy, and cows’ location (shade or sun) and their diurnal behaviors in a silvopastoral system of a subtropical climate, covering the four seasons.

2 CHAPTER I - A SYSTEMATIC REVIEW OF THE EFFECTS OF SILVOPASTORAL SYSTEM ON THERMAL ENVIRONMENT, BEHAVIOR, AND PHYSIOLOGICAL RESPONSES OF DAIRY CATTLE

Matheus Deniz[1], Karolini Tenffen de Sousa[1], Frederico Márcio Corrêa Vieira[2], Marcos Martinez do Vale[1], João Ricardo Dittrich[1], Maria José Hötzel[3]

[1]Laboratório de Inovações Tecnológicas em Zootecnia, Departamento de Zootecnia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil.
[2]Grupo de Estudos em Biometeorologia, Universidade Tecnológica Federal do Paraná, Dois Vizinhos, Paraná, Brazil.
[3]Laboratório de Etologia Aplicada e Bem-estar Animal, Departamento de Zootecnia e Desenvolvimento Rural, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil.

Correspondent autor:
Matheus Deniz;
ORCID: 0000-0001-8079-0070

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Abstract

Does the silvopastoral system (SPS) promotes a satisfactory thermal environment for dairy cattle to perform their natural behaviors and show a suitable thermoregulatory function? To answer this question, peer-reviewed, published articles, written in English that evaluated the effects and potential benefits of silvopastoral systems to dairy pasture-based system were used in a systematic review and bibliometric approach. We conducted a search using Web of Science and Google Scholar to identify key literature on effect of SPS on thermal environment, dairy cattle behavior, and physiology. The resulting articles (1448) underwent a 4-step appraisal process and result in 19 articles that fit our review criteria. Our review highlights different benefits of silvopastoral systems for grazing dairy cattle. For example, the SPS provides a better thermal environment than treeless pasture, that increases feeding behaviors; further, dairy cattle in SPS showed reduced drinking events, surface temperature, and respiratory rate. However, some evaluated variables (n=9) related to animal behavior and physiology responses showed unclear results; i.e., no difference between the SPS and treeless were found. Furthermore, behaviors associated with lie down (e.g., idling, and rumination), and physiological responses (e.g., milk production and heart rate) has been low explored in studies of SPS. In conclusion, we identified that the main effects of silvopastoral systems on the microclimate variables and thermal comfort indicators have a strong consistency of evidence to be beneficial for dairy cattle. However, the evidence regarding the effect of SPS on animal behavior and physiology responses is still scarce and unclear requiring further research in this field.

Keywords: agroforestry systems, biometeorology, heat abatement, livestock farming, milking, shade

3 INTRODUCTION

Livestock farming is an important user of natural resources (Leip et al., 2015); however, over the years, its advancement led to several negative impacts throughout ecosystems (Sullivan et al., 2017). The increasing challenge of climate changes (IPCC, 2021) associated with the intensification of heat waves throughout the world (Carvajal et al., 2021), resulted in a rise of scientific papers to assess mitigation strategies for the environmental impacts of livestock farming. However, the number of publications is expanding in a fragmented way, and the task of accumulating knowledge becomes more complicated. Thus, scientific mapping is becoming an essential activity for scholars from all scientific fields. The synthesis of conclusions from the literature is required to offer a debate of sustainable production; since different governments show interest in producing farm commodities in a sustainable way (Rasmussen et al., 2017).

The adoption of management practices such as pasture-based systems can be the best alternative to use natural resources profitably and safely for the environment (England et al., 2020; Machado Filho et al., 2021). Pasture-based systems can allow animals the opportunity to express their natural behavior. Nevertheless, one of the biggest concerns in pasture-based systems is the climatic conditions (Herbut et al., 2018), as animals are constantly subjected to a large environmental variability. In heat stress, the animals activate mechanisms for dissipating heat load, like changing their behaviors and physiology. Some negative effects associated with heat stress are the increased of body temperature (Sejian et al., 2018; Lees et al., 2019), and respiratory rate (Herbut et al., 2018); further, decreased of feed intake (Chang-Fung-Martel et al., 2021), production and quality of milk (Blanco-Penedo et al., 2020), and fertility (Sejian et al., 2018); in extreme cases, the heat stress can result in the animal' death (Vitali et al., 2015).

The negative effects of heat stress on dairy cattle can lead to an economic loss around $900 million per year for US dairy industry if cows were not cooled (St-Pierre et al., 2003; Ferreira et al., 2016). Because of this, it is important to provide elements to mitigate adverse situations (Herbut et al., 2018, 2019; Sejian et al., 2018). Besides to improve animals' welfare, the general public prefers production systems that promote heat abatement for farm animals (Cardoso et al., 2018). While the heat abatement for housed cows is provided using convective (e.g., fans; Vieira et al., 2021) and evaporative cooling systems (e.g., sprinkers; Tresoldi et al., 2018) in pasture systems, the silvopastoral systems has been highlighted as an important element to mitigate the effects of environmental factors (Deniz et al., 2019; Magalhães et al., 2020; Karvatte et al., 2021). The aim of this review was to provide a critical and systematic evaluation of the scientific literature on the effects of silvopastoral systems on physical environment, thermal comfort indicators, behavior and physiological responses of dairy cattle. Furthermore, the rationale for this review was to generate useful information based on research with silvopastoral for dairy cattle to help decision-making in future studies involving dairy cattle.

4 METHODOLOGY

This review was based on the strategy of Population, Intervention, Comparison and Outcome (PICO). The acronym PICO is based on the model used in Evidence-Based Practice recommended for systematic reviews (Santos et al., 2007). Thus, we define a question to be answered: “Does the silvopastoral system promotes a satisfactory thermal environment for dairy cattle to perform their natural behaviors and show a suitable thermoregulatory function when compared to treeless pasture?”. This review was conducted in accordance with the guidelines of Preferred Reporting Items for Systematic Review and Meta-analysis protocols (PRISMA; Moher et al., 2015).

4.1 SEARCH STRATEGY

Peer-reviewed articles, written in English, published before June 2021 were systematically reviewed. The systematic searches were conducted using the Web of Science and Google Scholar databases with the integration of Boolean operators (i.e., AND, OR, NOT) to string together words or phrases, as well as wildcard truncations (denoted as " ") to designate a range of possible word forms. The “*” symbol was employed to account for alternate spellings (e.g., American versus British English). All the search terms are shown in Table 4-1.

Table 4-1 Population and outcome search term strings used for the final search in the systematic review.

Acronym	Search string
Interventions	(“silvopastoral system” OR silvopastoral OR silvopasture OR agroforestry OR “tree on pasture” OR “crop-livestock-system” OR agrosilvopastoral OR shad*)

Population	(cattle OR cows OR calves OR heifers) AND (dairy OR milking)
Outcome	(“thermal comfort” OR microclimate OR “thermal environment” OR thermal stress) AND (“thermal indices” OR “thermal index”) AND (behavio* OR physiology OR thermoregulatory)

4.2 STUDY INCLUSION CRITERIA AND SCREENING

We selected experimental studies that related the effects of silvopastoral system on microclimate variables, thermal comfort indicators, behavior, and physiology responses of dairy cattle. To confirm the potential benefits of silvopastoral systems, we considered studies that compared SPS to treeless pasture and/ or shaded and sunny areas within the SPS. Exclusion and inclusion criteria for the systematic review were developed a priori and agreed upon by all co-authors.

Results from the searches were pooled, and initially, duplicate results were excluded. Articles were then selected based upon a 4-step screening and appraisal process (Figure 4-1): Step 1. Publications written in a language other than English and other publications including thesis, book, book chapter, conference proceedings, and reports were removed. The articles remaining were scanned to filter out irrelevant results (e.g., the literature clearly pertaining to animals other than the dairy cattle). Step 2. Titles and abstracts were evaluated to identify and remove additional articles not relevant to the topic and out of interest (e.g., housed animals, dairy-herd economics, artificial shade, ecosystems services of the silvopastoral system, effect of silvopastoral systems on pasture, etc.). Step 3. In this step, titles and abstracts were evaluated to identify and remove additional articles that did not use dairy cattle (e.g., articles addressing beef cattle, sheep, and buffalo). Step 4. Finally, review articles were removed, and full texts of the remaining articles were read in detail. Articles containing experimental research were excluded if the experiment itself did not address the relationship between the microclimate variables and/ or thermal comfort indicators, behavior, and/ or physiology of dairy cattle in a silvopastoral system. In addition, articles about the mathematical models were excluded if parameters analyzed were sourced from other literature, or if insufficient information pertaining to real-world data collection was provided to permit recalculation of model parameters.

The articles remaining at this stage were included in the systematic review, and in multiple sections if they described more than one relevant effect. To provide a comprehensive overview of the literature, no additional restrictions were placed upon publication year, study type, sample size, journal, or overall quality. We excluded conference proceedings (both papers and abstracts), as well as book chapters, as we could not be certain that these sources had been peer-reviewed. We also excluded literature in languages other than English, as we were unable to critically assess the methods and evaluate the results. We are unable to determine to what extent these exclusions affected the conclusions of this review.

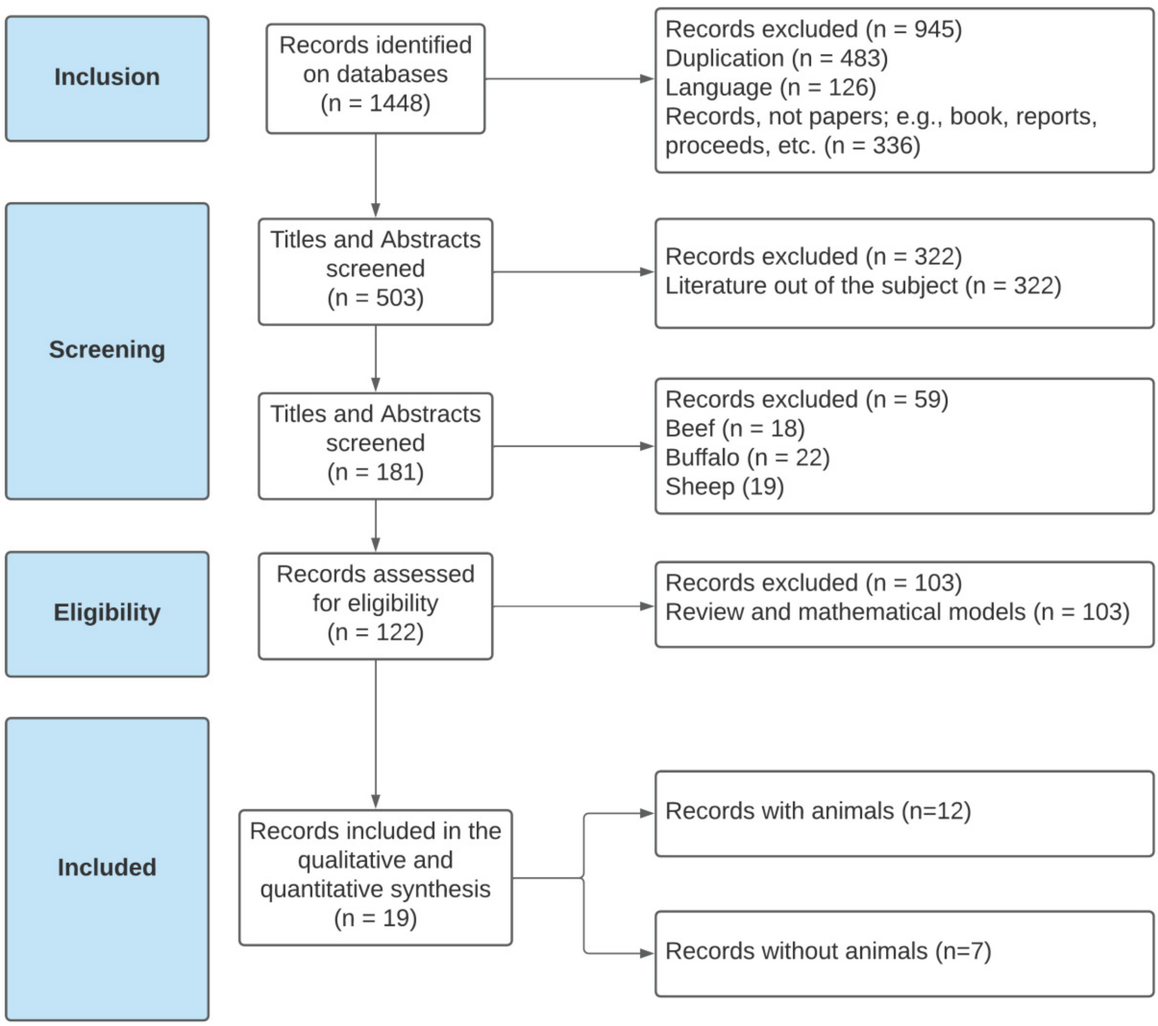

Figure 4-1. Flowchart following PRISMA guidelines (Moher et al., 2015), showing the total number of publications identified and the number of publications filtered at each stage of the selection process from the systematic review.

4.3 DATA EXTRACTION

Two investigators independently screened full texts for all the articles. Inter-observer reliability for data extraction (for all categories except for authorship and publication year) was tested, and we obtained 100% agreement. The bibliometric analysis was performed by the Bibliometrix R package (Aria and Cuccurullo, 2017; R Core Team, 2021). The Bibliometrix determines the intellectual structure of scientific domains using the network analysis with multiple correspondence analyses on keywords, titles, and abstracts of the articles. To determine whether the choice of search terms in the databases was appropriate, a word cloud containing the 25 most cited words in the abstract (threshold of frequency was >30%) was built. In addition, the co-occurrence network and link (relationship between knowledge areas) between the words used on abstracts of the articles (Cobo et al., 2011), and a thematic map of the most relevant words used on the abstract were built. For interpretation purposes, the size of the label and circle of a term is determined by its weight, i.e., the number of times the term was used in the articles (Van Eck and Waltman, 2014). The links show the relationship between the knowledge areas, i.e., the closer terms have a stronger relationship.

We recorded the system evaluated, characteristic of a silvopastoral system (tree arrangement and density), shaded area by animal, climate of the studied region, period of data collection, season, breed of cattle, number of animals, and variables evaluated (microclimate variables, thermal comfort indicators, behavior, and physiology responses). Also, we registered the outcome measure(s) and the predominant direction of the results (England et al., 2020; Beaver et al., 2021). To represent the direction of the results we used "+" indicating an increase of the evaluated variable, "-" indicating a decrease of the evaluated variable, and "=" indicating no difference at the threshold (p-value) used by authors. Then, the predominant direction (PD) of the results (positive, negative, and no difference) was calculated according to equation (1) proposed by Harrison et al. (2014) and modified from England et al. (2020).

$$\text{Predominant direction} = \frac{\sum(\mathrm{PI}) - \sum(\mathrm{NDE} + \mathrm{ND})}{\text{total number of studies evaluated the evidence}} \qquad (1)$$

Where: PI is the number of articles that found an increase of the evaluated variable when compared to treeless pasture; NDE is the number of articles that found no difference between the systems; and ND is the number of articles that found a decrease of the evaluated variable. For the PD interpretation purposes, if was >0, then the results were interpreted as predominantly positive; if was < 0, then the results were predominantly negative or unclear. Furthermore, to assess whether the results found by the studies included in this review were consistent, with the direction of the results, we determined the consistency of evidence according to equation (2)

$$\text{Consistency of evidence} = \frac{number\ of\ studies\ that\ found\ the\ evidence}{total\ number\ of\ studies\ that\ evaluated\ the\ evidence}\ x\ 100 \qquad (2)$$

The consistency of evidence was classified in three levels: (†) slight association (≥33), (††) moderate association (range: 34 to 59), and (†††) strong association (range: 60 to 100). Also, the level (*) was created, which means changing in association dependent on trees density.

5 RESULTS

Of the 19 articles included in this review, the years of publication ranged from 2010 to 2020. In total, 12 articles (63%) involved animals, and 7 (37%) are environmentally based articles (without animals). The studies were carried out in Brazil (n=18) and Belgian (n=1), and the main characteristics considered for the experimental trials are shown in Table S2. An examination of these characteristics (Table S2) demonstrated that the studies selected did not use the same methodology, so we discarded the meta-analysis.

The word cloud of the abstracts (Figure 5-1) highlighted five words that were used with a frequency greater than 40% (shade – 69%, temperature – 65%, thermal – 53%, cows – 48%, and systems – 42%). The use of other words ranged from 19% to 37%. It is important to note that search terms that we used in the search were consistent with the words that appear in the abstracts of the 19 articles included in this review. The co-occurrence network analysis and links among the most relevant words used in the abstracts are shown in Figure 5-2. The smallest circles represent themes that have been explored in fewer articles.

Figure 5-1 Word cloud generated using the 25 most frequently used words in the abstract of the 19 articles included in this review. The words appearing in larger type were used most frequently.

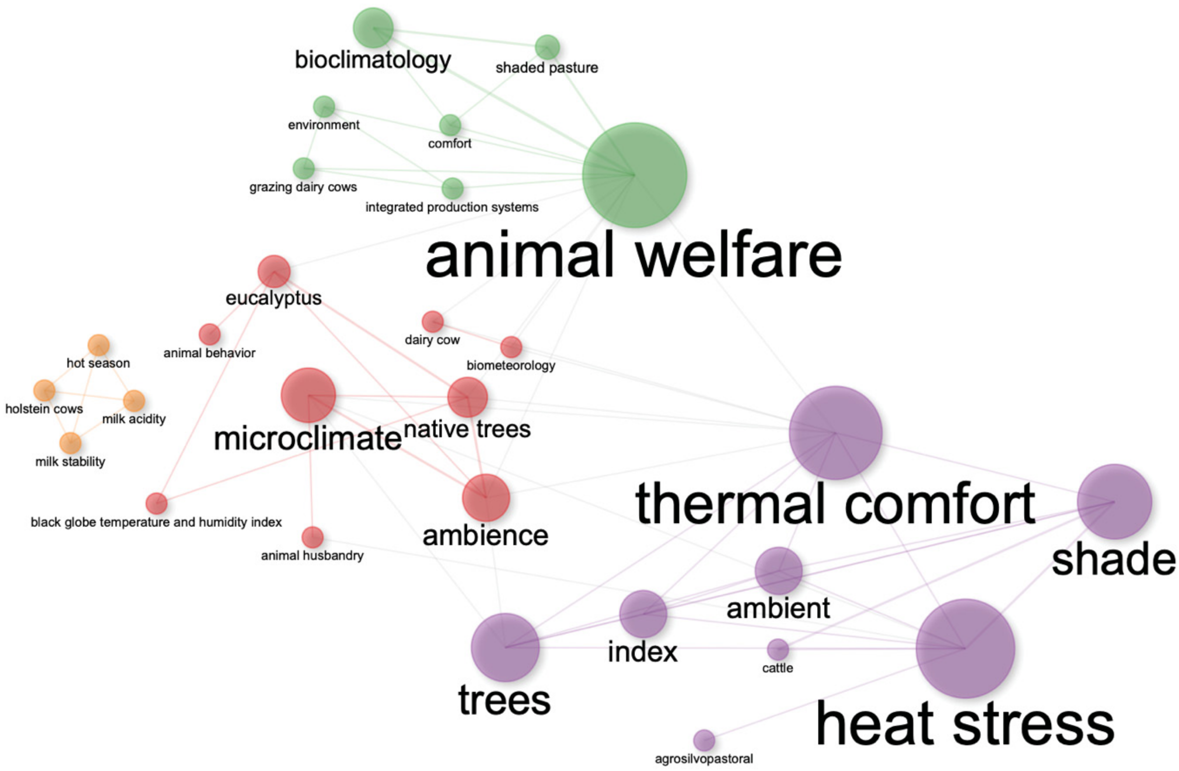

Figure 5-2. Co-occurrence network of the words on the abstracts from the 19 articles included in in this review. The size of the label and circle is determined by the number of times the word was used, and the links show the relationship between the knowledge area; the closer word have a stronger relationship.

Of the 19 articles included in in this review, 8 (42%) compared silvopastoral systems to treeless pasture, 8 (42%) compared different tree arrangements (different trees density) to treeless pasture, and 3 (16%) compared shaded and sunny areas within the silvopastoral systems. In addition, 17 articles (89%) performed measures during diurnal period (range: 5:30h to 18h) and 2 collected data during 24h. In general, the duration of evaluation period ranged from 3d to 12d. All articles (n=19) evaluated microclimatic variables, 7 (89%) evaluated thermal comfort indicators, 10 (53%) evaluated animal behavior, and 7 (37%) assessed dairy cattle's physiological responses. Of the 12 articles with animal, the majority (75%) used up to 20 animals (range: 8 to 20). In summary, 6 articles (50%) evaluated lactating cows, 2 (17%) dry cows, and 4 (33%) heifers. Two articles (17%) did not evaluate animals' behavior, and only 5 articles (42%) determined the shaded area by animal (range: 2 to 10.5 m²).

5.1 MICROCLIMATE VARIABLES AND THERMAL COMFORT INDICATORS

Of the 19 articles included in this section, all evaluated microclimatic variables and 17 articles (89%) evaluated thermal comfort indicators. In general, 7 articles used only the temperature humidity index (THI) to estimate the animals' thermal comfort and 2 articles did not consider any thermal comfort indicators. The predominant direction of the results and consistency of evidence of the effects of silvopastoral systems on microclimatic variables and thermal comfort indicators are shown in Table 5-3. In summary, 5 articles (75%) that evaluated microclimate found lower air temperature values in silvopastoral system; whereas, only 1 articles found higher relative humidity values in treeless pasture. In relation to the wind speed, 3 articles (60%) found lower values in silvopastoral systems; however, 1 article highlighted the influence of tree density on wind speed. In addition, 3 articles (75%) found lower black globe temperature values in silvopastoral systems. Only 4 articles (21%) measured soil surface temperature; of these 2 articles (50%) found a positive correlation between black globe temperature and soil surface temperature, while 1 article (5%) showed a clear relationship between these variables and the behavior of grazing cows. Two articles did not find a difference in THI values between silvopastoral systems and treeless pasture; however, another 2 articles highlighted the influence of tree density on THI values. Articles that used thermal comfort indicators that consider solar radiation (n=9) found lower values in the silvopastoral systems than treeless pasture.

Table 5-1 Summary of literature review of the effect of silvopastoral system on microclimate variables and thermal comfort indicators when compared to treeless pasture. Total number of articles (N), results that showing an increase (N+), results that did not differ (N0) or results that showing a decrease (N-), predominant direction (PD) of results, and consistency of evidence for each variable evaluated.

Microclimate Variables	Literature review					PD	Consistency of evidence[2]		
	N	Reference[1]	N+	N0	N-		N+	N0	N-
Air Temperature	8	3,4,7,9,10,13,15,16	0	3	5	0.3		††	†††
Relative Humidity	7	3,4,7,9,10,13,16	2	4	1	-0.4	†	††	†
Wind Speed	5	7,9,13,15,16	0	2	3	0.2		††*	†††
Black Globe Temperature	4	9,10,13,15	0	1	3	0.5		†	†††
Soil Surface Temperature	4	5,9,11,16	0	0	4	1.0			†††
Thermal comfort indicators	**Literature review**					**PD**	**Consistency of evidence[2]**		
	N	Reference[1]	N+	N0	N-		N+	N0	N-
Temperature Humidity Index	7	3,4,7,13,15,16,18	0	4	3	-0.1		††	††
Black Globe Humidity Index	5	9,10,13,15,18	0	0	5	1.0			†††
Radiant Thermal Load	4	13,15,18	0	0	4	1.0			†††

[1]number of the article defined in Table S2; [2]slight [†], moderate [††], and strong [†††] association; [*] dependent on trees density.

5.2 ANIMAL BEHAVIOR

The drinking events was the only behavior variable that has strong consistency of evidence that decrease on silvopastoral system. Most behaviors evaluated showed unclear results; this fact was related to the negative predominant direction of the results since the articles did not find the difference between the pasture systems evaluated (Table 5-2).

Table 5-2 Summary of literature review of the effect of silvopastoral system on animals' behavior when compared to treeless pasture. Total number of articles (N), results that showing an increase (N+), results that did not differ (N0) or results that showing a decrease (N-), predominant direction (PD) of results, and consistency of evidence for each variable evaluated.

Behavior parameters	Literature review					PD	Consistency of evidence[2]		
	N	Reference[1]	N+	N0	N-		N+	N0	N-
Grazing	6	1,3,4,7,9,12	3	1	2	0.0	††	†	†
Resting	7	1,3,4,7,8,9,12	1	3	3	-0.7	†	††	††
Rumination	7	1,3,4,7,8,9,12	4	3	2	-0.1	††*	††*	†
Resting Lying	3	1,9,12	1	1	1	-0.3	†	†	†
Rumination Lying	3	1,9,12	1	2	0	-0.3	†	†††*	
Drinking events	6	1,3,4,7,9,12	0	2	4	0.3		†	†††

[1]number of the article defined in Table S2; [2]slight [†], moderate [††], and strong [†††] association; [*] dependent on trees density.

5.3 PHYSIOLOGICAL RESPONSES

Of the 7 articles that evaluated physiological responses, 3 variables shown a strong consistency of evidence that decrease on silvopastoral system (Table 5-3). Two articles (29%) found a decrease on the animals' surface temperature (~2.2 °C), 4 reported decreased on respiratory rate (~13 breaths/min.; range: 4.6 to 20 breaths/min.) and 2 reported a decreased-on panting score (range: 0.9 to 1.75). Of the 5 articles that evaluated animals' internal temperature, 3 (60%) did not found a significant difference between silvopastoral systems (~38.9 °C) and treeless pasture (~39.1 °C). Also, 2 articles that evaluated heart rate and other 2 that evaluated milk production, did not found a significant difference between the pasture systems.

Table 5-3 Summary of literature review of the effect of silvopastoral systems on physiological responses when compared to treeless pasture. Total number of articles (N), results that showing an increase (N+), results that did not differ (N0) or results that showing a decrease (N-), predominant direction (PD) of results, and consistency of evidence for each variable evaluated.

Physiological parameters	Literature review					PD	Consistency of evidence[2]		
	N	Reference[1]	N+	N0	N-		N+	N0	N-
Surface Temperature	2	7,10	0	0	2	1.0			†††
Internal Temperature	5	1,4,6,10,12	0	3	2	-1.0		†††	††
Respiratory Rate	4	1,6,7,12	0	0	4	1.0			†††
Heart Rate	3	1,6,12	0	2	1	-0.3		†††	†
Milk production	2	1,10	0	2	0	-1.0		†††	
Panting Score	2	1,6,	0	0	2	1.0			†††

[1]number of the article defined in Table 2; [2]slight [†], moderate [††], and strong [†††] association; [*] dependent on trees density.

6 DISCUSSION

A key finding of our review was that the effect of silvopastoral system (SPS) on biophysical variables evaluated were majority (57%) positive; for example: the SPS provides a better thermal environment than treeless pasture, promote an increase on grazing and rumination behaviors; further, dairy cattle showed low drinking events, surface temperature, and respiratory rate. However, it is important to highlight that, unlike other studies, our review also evaluated the possible negative and unclear effects of silvopastoral systems. The negative predominance direction of the results for some variables (e.g., temperature humidity index, lying behavior, and heart rate) was associated with unclear results, i.e., the authors did not find a significant effect of the silvopastoral system on the evaluated variables. The unclear results can be related to several factors, such as the seasons in which the study was performed, tree, and methodology of data collection. We note that the research efforts on silvopastoral systems as a strategy for heat abatement for dairy cattle are limited. This fact was supported by the number of articles (n=19) that fitted in our review criteria; which in turn, did not allow us to perform a deeper analysis to understand the variability on the results. Other interesting fact is the majority of studies was performed in Brazil, while studies carried out in other countries were eliminated due to not integrating the relationship of the physical system with animals' response, since most were focused on evaluating artificial shade (e.g., Sharpe et al., 2020) and ecosystem services (England et al., 2020).

Understanding the benefits of silvopastoral systems can influence the adoption of more sustainable animal production systems from an animal and environmental point of view. Once rearing animals on pasture areas allow them to express their natural behaviors (Beaver et al., 2021; Mee and Boyle 2020), and improve their positive affective states, as an increase in the number of affiliative interactions between cows (Améndola et al., 2016). Furthemore, as suggested by the Kohari et al. (2007) trees on pasture provide a good environmental enrichment object that satisfies cattle's potential needs of grooming; which in turns, increase the levels of animal welfare (McConnachie et al., 2018). By including elements in the rearing system, we are giving to the animals the opportunity to make choices based on their previous experiences, or emotional states (positive or pessimistic). Furthermore, the silvopastoral system can lessen the negative effects of social hierarchy; since it includes another resource (shade) for heat abatement, so animals can choose between drinking water or using shade, depending on their motivation or the presence/absence of another animal in certain areas (Deniz et al., 2021b). However, to achieve improvements in pasture-based livestock, it is necessary for research to include animal assessments. Since the positive effect of the silvopastoral system on the thermal environment is widely known, but the effects on quality of life of animals, as the emotional states and the use of different thermoregulatory resources (shade and water) has been little explored.

6.1 MICROCLIMATE VARIABLES

We found a strong consistency of evidence that the silvopastoral system had the lowest air temperature. Although silvopastoral systems are often associated with better comfort conditions for grazing animals, it is not possible to say that this is an absolute truth, since 3 articles did not find difference between silvopastoral system and treeless pasture. Our findings suggest a moderate consistency of evidence for unclear results of relative humidity in silvopastoral systems. Researchers are constantly evaluating the physical environment to indicate different levels of stress on farm animals. Air temperature is directly related to the animals' thermoregulation, involving the chemical, physical and biological mechanisms of the animals to deal with thermal fluctuations in the environment (Cossins, 2012). However, the relationship between relative humidity and heat stress is not clear, as the ability of animals to withstand heat is inversely proportional to the relative humidity; when the relative humidity is high, farm animals generally have more difficulty to dissipate the internal heat load by respiratory evaporative loss (de Castro Júnior and Silva, 2021).

Although our results suggest an unclear effect of the silvopastoral system on the relative humidity, this may be associated with the morphological characteristics of the trees and the planting density (Karvatte et al., 2016). This can be explained by the windbreak function provide by trees on pasture systems. A decrease on wind speed was observed with the increasing on tree density (from 227 to 357 trees/ha; Oliveira et al., 2017), while there was no difference in wind speed in systems with lower tree density (100 trees/ha; de Sousa et al., 2021a) compared to treeless pasture. However, high trees density can provide uncomfortable environments for animals, as the low wind speed reduces the animal's capacity for thermal exchanges with the environment (Fournel et al., 2017). It is important to highlight that even the wind speed being important for the animals' thermoregulation, only 5 studies (Souza et al., 2010; Oliveira et al., 2017; Deniz et al., 2019; Vieira et al., 2020; de Sousa et al., 2021b) evaluated this variable. Thus, we suggest that new studies include the evaluation of wind speed so that we can have better clarity of the effects of the silvopastoral system on the thermoneutral conditions for dairy cattle.

There was a strong consistency of evidence that the silvopastoral system had a low black globe temperature and soil surface temperature, favoring thermal exchanges by conduction and convection (Nordlund et al., 2019). Both variables had the greatest positive predominance direction probably because they are correlated (de Sousa et al., 2021b). Trees provide the most effective shade because they combine the sun protection with the radiation sink effect created by cool leaves evaporating moisture (Armstrong, 1994), also decreasing the soil surface temperature (Deniz et al., 2019; de Sousa et al., 2021b), as the environment thermal load decreases (Souza et al., 2010; Oliveira et al., 2017; Magalhães et al., 2020).

6.2 THERMAL COMFORT INDICATORS

Of the 17 articles that addressed thermal comfort indicators, 7 did not consider animals and focused only on environmental evaluations. On livestock farming is common use thermal comfort indicators to infer the animals' thermal condition. Also, the thermal indicators help to interpret the complex interactions between the physical (environment) and biological (animal) components of the environment, which must be interpreted according to the individual characteristics of each animal species (Sejian et al., 2018; Lees et al., 2019). Farm animals are homeothermic and respond differently to environmental variations through behavioral (Herbut et al., 2020) and physiological response (Sejian et al., 2018). Therefore, it is important to consider the animals as agents of the systems, so that we can evaluate the relationship of the environmental conditions with behavioral and physiological responses of animals, to determine the real animals' thermal comfort on different production systems.

Of the articles selected in this review, only 1 (de Sousa et al., 2021b) used a cold stress indicator (wind chill temperature). According to de Sousa et al. (2021b), in the winter of the subtropical climate, dairy cows can be exposed to thermal challenged conditions during the night facing thermal sensation below the lower critical threshold (4°C for dairy cattle). Cold also can be a thermal stress and its effects are delayed and prolonged (Cox et al., 2016); furthermore, the intensity and duration of cold waves increase cattle mortality (Morignat et al., 2018). However, the evidence identified in this review is mainly related to the benefits of shade areas to heat abatement for animals; while the silvopastoral systems also have the potential to provide shelter from the cold and wind.

Our findings showed that the silvopastoral system promote the lowest values of thermal comfort indicators that considerer solar radiation, indicating a better thermal environment for dairy cattle than treeless pasture. On the other hand, results for temperature humidity index were unclear, as 4 of the 7 studies did not find a significant difference between the silvopastoral systems and treeless pasture. The temperature humidity index is one of the most thermal comfort indicators used in farm animals (see reviews: Herbut et al., 2018; Sejian et al., 2018). However, one disadvantage of this thermal index is that only consider two environmental variables: air temperature and relative humidity (Sejian et al., 2018). It is important to emphasized that the variable solar radiation is the mainly environmental factor that led to heat stress on animals raised on pasture (Magalhães et al., 2020; Deniz et al., 2021a).

6.3 ANIMAL BEHAVIOR

The effects of the silvopastoral system on the behavior of dairy cattle were unclear, since the consistency of evidence (range: from moderate to strong) was associated with no difference between silvopastoral systems and treeless pasture. Of the 12 studies with animals, 2 (De Abreu et al., 2020; Martins et al., 2021) did not evaluate animal behavior and 2 (Carnevalli et al., 2020; Deniz et al., 2020) did not address thermal comfort indicators. The last two studies used microclimatic variables to assess the effect of the thermal environment on the behavior of cattle. In addition, 6 studies considered only the temperature humidity index to assess the effect of the thermal environment on animals. Although it is already known that thermal comfort indicator that consider solar radiation are more important to classify the behavior of grazing animals to access shaded areas (Deniz et al., 2021a; Volpi et al., 2021).

Thermal environment can induce behavioral changes, such as decrease on time spent lie down, food intake, and reproductive behavior; further, increase the time spent in standing rest, aggressive behavior, and water intake (see reviews: Polsky and von Keyserlingk, 2017; Herbut et al., 2020). As an adaptive strategy to environmental challenges during the summer, dairy cattle stay longer in shaded areas (Van Laer et al., 2015; Deniz et al., 2020), as these areas present better environmental conditions (Karvatte et al., 2016; Oliveira et al., 2017; Pezzopane et al., 2019; Magalhães et al., 2020) than treeless pasture. However, in winter, cows can also use the shade when motivated, either to protect themselves from the sun in the middle of the day or to rest (de Sousa et al., 2021b). An additional issue is that further studies must consider compensatory strategies adopted by the animals, such as grazing at night (Skonieski et al., 2021), but 89% of the studies performed collections only during the day. Even though, it is widely known that the silvopastoral system is more effective during the day to promote sun protection and thermal comfort; perhaps it is important that future research increases the observation period, allowing for greater clarification on the behavior of animals.

Drinking events was the only variable that had strong consistency of evidence that decrease on silvopastoral system. In silvopastoral system animals have access to shade and water; these two resources are important for the maintenance of body homeostasis as they help in thermal exchanges (Sejian et al., 2018). The importance of providing shade is noticed since cows spend more than twice as much time in shaded areas instead of visiting the water trough (Deniz et al., 2020), even that the need for water under heat stress increases by up to two times due to water losses through evaporation. In contrast, on the absence of shade or when the shade is not provided in sufficient quantity and quality, the cattle spend longer at the water through (Vizzotto et al., 2015; Vieira et al., 2020; de Sousa et al., 2021b). Thus, the milder microclimate provided by the silvopastoral system may be responsible for reducing the water intake. Systems that require less water and use it more economically are essential for greater sustainability of the activity.

Although lying behaviors (idle and rumination) are an important indicator of comfort and animal welfare (Tucker et al., 2020), few studies have evaluated them. Shaded areas are more attractive for cattle to lie down (Vizzotto et al., 2015; Deniz et al., 2021b), as lying on surfaces with milder temperatures favors latent and sensitive heat exchange (Gebremedhin et al., 2016; Nordlund et al., 2019). However, only 1 study (de Sousa et al., 2021a) found a clear relationship between the soil surface temperature and the lying behavior of cows. de Sousa et al. (2021a) highlighted that this occurs in the winter condition, in which the animals possibly went through conditions of cold stress at night and preferred to lie down in the sun during the hottest hours of the day. Heat exchange (gain or loss) by conduction is a recent research topic in dairy cattle, and studies have focused on assessing the moisture and conductivity of surfaces in confined systems (Gebremedhin et al., 2016; Nordlund et al., 2019). Meanwhile the effects of soil surface temperature (de Sousa et al., 2021b) and precipitation (Thompson et al., 2019) on lying behavior of dairy cattle has been less explored in pasture areas. This knowledge is not clear, as moist soils have better conductivity and lower temperature than dry soils (Zimmer et al., 2020); however, cows avoid lying down on wet surfaces because it is less comfortable to rest (Schütz et al., 2019). The research forward should address effort to assess soil temperature, moisture, and conductivity in pasture systems, as these factors can interfere with the thermodynamics of pasture-raised dairy cattle.

In this review, only 1 study evaluated the aggressive interaction among animals. Vizzotto et al. (2015) found more events of aggressive interactions around the water trough among cows keep without access shade. However, this study did not perform a deep analysis on the social behavior, like determining the social categories. Social hierarchy also plays a role in the harmful effects of heat stress, and it is already known that the use of resources available on the environment depends of the animal' social position (de Sousa et al., 2021c; Deniz et al., 2021b). Since dominant cows are more likely to use shaded areas than subordinate; in contrast, intermediate and subordinate cows are more likely to drink water (Deniz et al., 2021b). In addition, indoor-housed cows with low competitive success at the drinker shifted their drinking behavior on hot days to avoid the drinker during times of high competition (McDonald et al., 2020). The same authors suggested that the measures of competition may be a practical value in deciding when to provide cooling. Future research should continue to investigate how the social categories of dairy cattle influences heat stress coping strategies and competition for access heat abatement resources in pasture areas.

6.4 PHYSIOLOGY RESPONSES

When behavioral changes are not enough to cope with the effects of heat, the physiological responses are activated (Sejian et al., 2018; Lees et al., 2019; Herbut et al., 2020) as an attempt to keep the homeostasis without damage to the normal animals' body function. In the silvopastoral systems the variables surface temperature, respiratory rate, and panting score were lower than treeless pasture. However, one of the problems to measure body surface temperature is the highly sensitive to heat (Kadzere et al., 2002), but the body surface temperature is a non-invasive measuring and have a positive correlation with respiratory rate (Vieira et al., 2020), which is one of the main heat dissipation mechanisms (Polsky and von Keyserlingk, 2017). In addition, the panting score is a robust low-cost method for assessing heat stress of cattle over a range of geographical locations and climatic conditions (Gaughan et al., 2010) further it is a viable alternative to using body temperature (Brown-Brandl et al., 2006).

Although no studies in this review found an increase in physiology responses on animals raised in silvopastoral systems; it is important to highlight that the variables internal temperature, milk production, and heart rate had strong consistency of evidence for unclear results. This can be explained due to the missing statistical difference between the silvopastoral systems and treeless pasture for these variables. Two factors may have contributed to this; firstly, may be related to the methodological approach used in those studies, such as the type of device and sampling strategies that can affect the interpretation of the outcomes (Tresoldi et al., 2020). Measuring internal temperature with a shorter time of interval (120 min) results in more accurate 24-h estimates of the mean value when compared with strategies that recorded it once to thrice daily (Tresoldi et al., 2020). Of the 5 studies that evaluated internal temperature three authors used sampling ranging from once to twice daily measurements. Secondly, the duration of heat stress that the animals were submitted may have been short or the period of data collection did not comprehend the animal's physiological response. It is already known that cattle show a time lag in their response to heat stress (St-Pierre et al., 2003; Herbut et al., 2018) and that summarizing weather across the week preceding the test-day usually explained milk traits better than shorter-term summaries (Hill and Wall, 2015). So, one way to better understand the effects of weather in some traits is moving mean weather measurements. Hill and Wall (2017) calculated moving means for weather data recorded over the 3 and 7d before and including the test date to investigate the effects of weather on feed traits of dairy cows. McDonald et al. (2020) when evaluating how heat stress affects the behavior of indoor-housed cows at the drinker calculated moving averages for daily mean and maximum THI over a 3d period (weather spanning the day behavior was measured plus the previous 2d). We call for future research into the animals' physiological responses raised on pasture that extends beyond these actual condition explorations and describing the conditions that the animals were submitted before the collection data. This approach will allow the researchers to identify if the animals were or not in heat stress before that collection data starts.

7 CONCLUSIONS

Our review identified that some measures of the physical environment (e.g., air temperature, soil surface temperature, black globe-humidity index, and radiant thermal load), behaviors (e.g., grazing, rumination, and drinking events), and physiology responses (e.g., surface temperature, respiratory rate, and panting score) had moderate and strong evidence to be improved in silvopastoral system. Although we did not find any negative effect of silvopastoral systems, some caution is required. The evidence to identify the effects of silvopastoral systems on animals' behavior and physiology responses were relatively scarce and unclear. The general lack of negative impacts could be real or may reflect a bias in the literature towards only publishing studies with positive impacts; anyway, reporting negative impacts is also important so that production systems can be improved of the cattle point of view. Furthermore, new studies are necessary to understand the effects of silvopastoral systems on the behavior and physiology of dairy cattle.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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9 CHAPTER II - AGE AND BODY MASS ARE MORE IMPORTANT THAN HORNS TO DETERMINE THE SOCIAL POSITION OF DAIRY COWS

Matheus Deniz[1,3], Karolini Tenffen de Sousa[1], Marcos Martinez do Vale[2], João Ricardo Dittrich[1]

[1]Graduate Program on Animal Science, Federal University of Parana, Curitiba, 80.035-050, Brazil.
[2]Department of Animal Science, Federal University of Parana, Curitiba, 80.035-050, Brazil.

[3]Corresponding author:
Matheus Deniz; ORCID: 0000-0001-8079-0070
E-mail: matheus-utfpr@hotmail.com;

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Abstract

The aims of this observational study were to (1) define which animal's phenotypic characteristics determine social position in the context of a commercial organic farm with mixed herd (horned and non-horned cows) and (2) determine the influence of social position on the time at the feeder. We took the following measurements from 27 dairy cows in lactation: body mass, age, body condition score, body length, withers height, distance between horns, horn circumference and length. Replacement and time at the feeder were recorded for one hour at the time of supplementation. Dominance value for each animal were calculated and the herd was divided into three social categories: dominant (D), intermediate (I) and subordinate (S). Age, body length and body mass influenced ($p<0.001$) dominance value of all animals. The presence of horn influenced ($p=0.034$) the dominance value of the I and S animals because it was a unique characteristic of these categories. Dominant (84.3%) and intermediate (75.2%) animals spend more time ($p<0.05$) at the feeder than the subordinate (59.5%); however, dominant animals tended ($p=0.093$) to spend more time at the feeder than the intermediate animals. The social position of an animal was influenced by its age, body mass and body length, and its social position influenced the time at the feeder.

Key words: animal behavior; applied ethology; dominance value; dyads level; social hierarchy.

10 INTRODUCTION

Social behavior plays an important role in an animal's life (Proudfoot and Habing 2015). To gregarious species, like bovines, social interactions are evolutionarily important to maintain their fitness relative to the environment in which they live (Mendl and Held 2001). Social position affects several behaviors, such as feed and water intake in groups (Coimbra et al. 2012; Bica et al. 2019a,b). As part of their repertoire of natural behavior, cows organize themselves into hierarchies according to their willingness and ability to fight for scarce resources. The social hierarchy is established through dominance relationships, which are defined on the basis of aggressive interactions between cows (Beilharz and Zeeb 1982; Kondo and Hurnik 1990). Characteristics, such as social learning, age, weight, and horns are relevant to the animals` competitive capacity and, consequently, to their social position (Bouissou 1972; Šárová et al. 2013).

Presence of horn is a phenotypic characteristic of several breeds of dairy cattle. However, there is a general concern about horned cows in the herd, due to the injuries and stress that horned animals may cause to others (Waiblinger et al. 2001). Also, horned cows are not safe for farmers to handle (Knierim et al. 2015). Thus, a common procedure in dairy farms is the dehorning of animals. However, over the last few years, the growing public awareness of practices in livestock production systems leads to a demand to be closer to an animal's natural environment (Hötzel et al. 2017; Yunes et al. 2017). To meet consumer expectations, organic production is a good option. Organic production systems are known for their effort to keep their animals under species-appropriate conditions. It should be noted that the typical expectation is to leave the animal in its natural state with horns, and this alternative is already being applied in organic production since calf dehorning is prohibited (Brasil 2011). Therefore, organic dairy farms must be suitable for handling horned animals, as applying good animal husbandry to adapt facilities and improve management conditions, it may not be necessary to subject animals to this unnecessary painful procedure.

In livestock, facilities and management may aggravate or mitigate, the effects of social hierarchy (Bouissou 1980; Greter et al. 2013; de Vries et al. 2015). To mitigate the effects of social hierarchy, facilities must allow enough space for all animals to perform their respective behaviors (Knierim et al. 2015; Lutz et al. 2019). When food resources are not available in sufficient quantity for all animals or the feeder does not have enough space for all animals get together, the dominant ones have priority access to food, making it more difficult for subordinate animals to reach the feeder (Takanishi et al. 2015; Aniano and Ungerfeld 2016). However, by increasing feeding space and using feed barriers, aggression between animals can be reduced (Huzzey et al. 2006), which will, in turn, improve cow eating behavior, and this will benefit subordinate cows (Rioja-Lang et al. 2012; Hetti Arachchige et al. 2014). More space allowance at livestock facilities can also benefit both horned and non-horned cows as the risk of injury is reduced as a result of a decrease in aggressive interaction (Lutz et al. 2019).

Age is already known to be an important factor in determining social status, and older animals are more likely to be dominant (Šárová et al. 2013; Bonanni et al. 2017). Along with age, body mass is a trait that also influences social status, with older animals being significantly heavier than younger ones (Šárová et al. 2013). However, while in males, there is a general agreement that the main evolutionary benefit of horns is related to competition for partners (for more information see: Preston et al. 2003; Bro-Jørgensen 2007), for female (dairy cows) no consensus has been reached on the presence of horns (Knierim et al. 2015) in the social hierarchy and the effects therefore in mixed herds (Bouissou 1972; Beilharz and Zeeb 1982). In addition, recent studies that explicitly deal with social behavior in horned herds compared to non-horned herds are scarce. In this study, we evaluated which phenotypic characteristics of an individual affect its social position within a group and dyads level. Thus, our objectives in this observational study were to (1) define which animal's phenotypic characteristics determine social position in the context of a commercial organic farm with mixed herd (horned and non-horned cows) and (2) determine the influence of social position on the time at the feeder.

11 MATERIALS AND METHODS

This study was approved by the Ethics Committee on Animal Use of the Federal University of Parana under protocol number 083/2018, and it was performed in accordance with the ethics of animal experimentation. The experiment was carried out at the "*Centro Paranaense de Referência em Agroecologia*" (CPRA), Parana state, in Southern Brazil (25º26'41"S, 49º11'33"W). The climate of the region is characterized as humid maritime temperate (Cfb) according to the Köppen's classification. The region has mild summers with an average annual temperature between 18 and 20°C (Alvares et al., 2013). At the farm, animals were raised permanently on pasture, mainly composed of plant species of *Axonopus spp., Brachiaria spp., Pennisetum spp., Arachis spp., Cynodon spp., Trifolium spp., Setaria spp., Desmodium spp. and Lolium spp*. The pasture area was divided into 65 paddocks of 2000 m^2, under Voisin's Rotational Grazing system (Pinheiro Machado, 2010) whereby animals were moved daily to a new paddock. Mineral salt and water were offered *ad libitum* in the paddock.

11.1 BEHAVIORAL OBSERVATION

11.1.1 Animals

All lactation cows at the farm participated in this study, as they already formed a stable social group. Furthermore, given that our experiment was conducted on commercial organic farm, the experiment had to be included in the daily routine of the farm. In total, 27 (11 primiparous and 16 multiparous) dairy crossbred (Jersey/ Holstein) cows in lactation [days in milk 128 (range: 82 to 400), and milk production of 18 ± 3 L/ day; (mean ± SD)] were observed during the supplementation period. Horned animals (n=7) with a mean length of 16.8 cm ± 4.2, presented an average of 34 ± 7 months of age, body mass average of 404 kg ± 52, body condition score between 2 and 3.5 and days in milk average of 180. Non-horned animals (n=20) presented an average of 63 ± 24 months of age, body mass average of 410 kg ± 42, body condition score between 2 and 3.5 and days in milk average of 163.

11.2 DATA COLLECTION

Behavioral observations occurred during 20 non-consecutive days between June and July of 2019 (winter in the southern hemisphere). The choice for this period was due to (1) time of the year with low pasture availability in subtropical regions; provide corn silage during winter to animals after milking was a standard management in CPRA; (2) as Holstein and Jersey cows are from European genetics, the environment influence on behavior can be minimized when the observation is performed on milder temperatures. To ensure that all animals were in the same condition, they were deprived of access to the feeding area until the last animal was milked (milking length 1h). After that, it was offered 20 kg of corn silage per cow (dry matter: 25%). Five days before the experimental period, we tested whether the amount of corn silage per cow would be sufficient. It was offered 15 kg of silage per animal for two days and 20 kg per animal for three days. At the end of this period (5 days), we observed that 20 kg of silage per animal was not restricted as the animals did not consume it all during the observation time.

Observations were performed at the feeding area and lasted for one hour, starting after morning milking (approximately 0800 h). The area was already known by the animals and had a rectangular shape with approximately 300 m^2, concrete floor, and wooden fences. The feeder (40 m long) was covered with fiber-cement tiles (3 m high). The corn silage was supplied with a linear space of 1 m/ animal (using only 27 m from the feeder), which was considered sufficient for all animals to have access to the feeder in the same way (DeVries et al. 2004). Although all animals had access to the feeder in the same way, the situation of providing unrestricted corn silage at the feeder with a linear space of 1 m/ animal is considered a competitive situation when compared to pasture area. Throughout the experimental period, the animals had *ad libitum* access to the water trough and mineral salt.

Each cow was identified with a number (1 to 27) painted on the lumbar with commercial animal marking crayon. The aggressive interactions were recorded continuously whenever they occurred, and time at the feeder was directly recorded by scan-sampling at one-minute intervals (Altmann 1974). To minimize systematic bias, all observations were performed by four trained observers from an elevated place outside the feeding area, who daily switched the specific behavior being evaluated (aggressive interaction or time at the feeder).

11.3 MEASUREMENTS

11.3.1 Animal characteristics

Before the beginning of the experimental period, measurements of animals were collected in order to define those that influenced the dominance value of the animals in the herd. Animal measurements were as follows: body mass, body condition score, body length, withers height, distance between the horns, horn circumference and length (Figure 11-1).

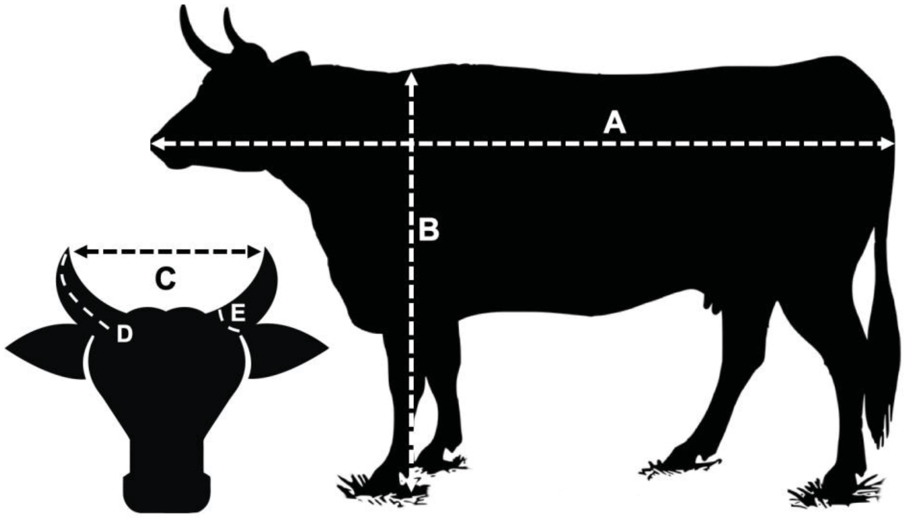

Figure 11-1. Schematic representation of animal measurements (A - body length; B - withers height; C - distance between horns; D - horn length; E - horn circumference).

11.3.2 Aggressive interaction – displacement at the feeder

All displacements at the feeder were recorded. A displacement was noted when a butt or a push from the actor (animal initiating the interaction) resulted in the complete withdrawal of the reactor's (animal losing the interaction) head from the feed rail. After collection, the data were analyzed with the aid of ETlog software (Deniz 2018), which in turn calculated the linearity index of the herd based on Landau (1951) and the dominance value (DV). The dominance value for each individual was calculated as a result of the sum of all relationships of each animal with all other animals within the group as described by Kondo and Hurnik (1990).

Social category was then assigned according to dominance value. As in this study, we chose to divide the animals into three social categories (dominant (D), intermediate (I) and subordinate (S)), so the divisor 3 was adopted for the equation 1. Social hierarchy (HS) was estimated by the distance between the highest (+ X) and the lowest (- Y) dominance value, plus 1 (corresponds to the dominance value zero), which determines the number of points in the range (equation 1) (see Coimbra et al., 2012).

$$SH = \frac{|\text{Distance between highest (+ X) and lowest (- Y) dominance value}| +}{3} \quad (1)$$

Animals with dominance value in the first tertile, i.e., those with the lowest values, including negative ones, were considered subordinate. Animals with dominance values in the second tertile were considered intermediate, and animals with dominance value located in the third tertile with higher positive values were considered dominant animals. Social category of each cow and its dominance value are shown in Table 11-1.

Tabela 11-1 Dominance Value (DV) and respective social category (SC; D = dominant, I = intermediate and S = subordinate) of each individual animal.

DV	SC	Animal	DV	SC	Animal
26	D	4	-4	I	15*
22	D	9	-6	I	3
22	D	13	-6	I	12
20	D	6	-6	I	23
18	D	22	-8	I	24
12	D	19	-9	S	18
9	D	25	-10	S	11
7	I	7	-11	S	1
5	I	8	-12	S	14
4	I	10	-16	S	16*
4	I	17	-18	S	5*
4	I	26*	-20	S	20*
0	I	2	-25	S	21*
-2	I	27*			

*Horned animals.

11.3.3 Time at the feeder

Observations began after the last animal entered the feeding area. Scan samples were recorded for each cow every minute, resulting in a total of 21,060 scan samples. A cow was considered eating corn silage at the feeder when: (1) her head was down at the feeder with the mouth at the silage; (2) her head raised the corn silage while chewing.

11.4 STATISTICAL ANALYSIS

All analyses (influence, descriptive, and confirmatory) were performed using the statistical software R (R Core Team, 2017).

11.4.1 Group-level analysis

Data analysis began with the assessment of normality assumptions using the Shapiro-Wilk test. Spearman's correlation was used to examine the relationships between all traits evaluated in the animals (age, number of lactations, body mass, horn presence, withers height, and body length) and dominance value. However, we only present the results of the characteristics that had significant correlation ($p<0.05$).

The characteristics that presented significant correlation with the dominance value were included as a fixed factor in a mixed model (Generalized Linear Models - GLM) to evaluate their relative influence on the dominance value. In the GLM, Gamma distribution, logarithmic bonding function, and a 95% confidence interval were used, defining animals as a random effect. With the aid of the maximum likelihood-Laplace approximation method in the lme4 statistical package (Bates et al. 2015), model adjustments were made. The power test was estimated using Type II chi-square tests and the fit of the model was given by a likelihood-test. The normality of the random facts was given by quartile plot means with a confidence interval of 95%.

In order to describe how much the weighted characteristics (age, body length, and body mass) influenced the response variable (dominance value), the regression β values were estimated. The regressor values were estimated with a generalized linear model, using Gamma distribution, logarithmic bonding function, and a 95% confidence interval. Linear regression was used to infer and verify the relationship among significant animal characteristics (age, body length, and body mass) in GLM models.

11.4.2 Horn presence

In order to confirm if the presence of horn influenced the dominance value of the lower-ranking animals (I and S), the data were submitted to a binomial distribution GLM and logistic bonding function at a confidence level of 95%. We compared the distance between the horns, circumference and horn length between the social categories (I and S), using a generalized linear model ($p<0.05$) with Gamma distribution and logarithmic bonding function. The power test was estimated using Type II chi-square tests and the fit of the model was given by a likelihood-test. The normality of the random facts was given by quartile plot means with a confidence interval of 95%. Distance between the horns, circumference, horn length, age, and body mass were submitted to the Spearman correlation test at the 95% level of confidence.

11.4.3 Time at the feeder

For eliminates the numerical (scans) discrepancy among social categories, feeder frequencies (%) were weighted according to the number of animals in each category (D - n = 7; I - n = 8; S - n = 12). Time at the feeder analysis was performed using a simple generalized linear model with Poisson distribution, logarithmic bonding function, and a 95% confidence interval. For the analysis, the social categories (D, I and S) were considered as independent variables, and the frequency of behavior at the feeder was considered a dependent variable. In order to confirm if the presence of horn influenced the time at the feeder of lower social categories (I and S), the data were analyzed using a simple generalized linear model with Poisson distribution, logarithmic bonding function, and a 95% confidence interval. Both models were adjusted using the maximum likelihood-Laplace approximation method in the lme4 statistical package (Bates et al. 2015).

11.4.4 Dyadic level analysis

To understand the dominance-relationships of pairs, we tested the influence of the quantitative within-dyad differences in age and body mass on dominance direction in pairs through a generalized linear mixed model. In each dyad (351 dyads), we randomly chose a focal cow and tested whether age and/or body mass superiority (or inferiority) affected the probability of her being dominant in the pair. The identities of the focal cow and the other cow entered the model as random factors. Finally, we used the chi-square test ($p<0.05$) to evaluate the effect of age and body mass on dominance value.

12 RESULTS

12.1 GROUP-LEVEL

During this study, each animal was able to relate to 26 other individuals, leading to a total of 702 possible pair combinations. In so doing, 2324 displacement at the feeder were recorded. We assumed that the social hierarchy of the herd was linear (linearity index = 0.82; $p<0.001$). A difference was observed ($p<0.001$) in the frequency of actor and reactor by social category. Dominant animals were actors (49.05%) more frequently than intermediate (38.2%) and subordinate (12.7%) animals. Subordinate animals were the most frequent reactor (47.2%), followed by intermediate (42.2%) and dominant (10.5%) animals.

There was an influence ($p<0.001$) of animal's characteristics on the dominance value (Figure 12-1). Dominant animals were the oldest of the three social categories (D – 6.3 years ± 0.78) with the highest body mass (D – 444.6 kg ± 10.69). Intermediate animals tended ($p=0.098$) to be older (I – 4.5 years ± 0.48; S – 3.5 years ± 0.42) and heavier (I – 406.9 kg ± 11.85; S – 377.5 kg ± 10.81) than subordinate. However, there was a different ($p<0.05$) in body length among social categories (D – 220 cm ± 2.81; I – 213.6 cm ± 1.85; S – 206.4 cm ± 2.33).

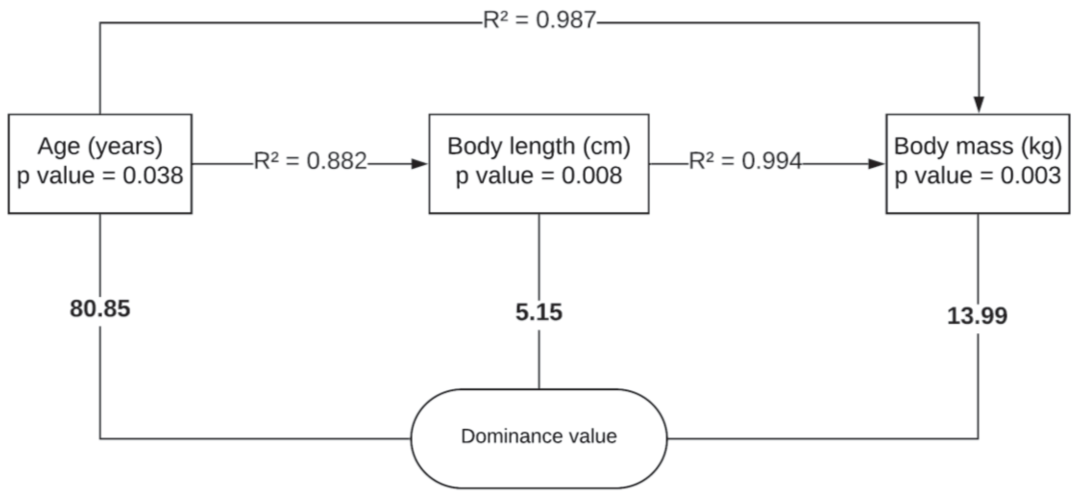

Figure 12-1 Representation of the statistical model with the influence of animal characteristics on the dominance value. Arrows show the regression determination coefficient (R^2) relative to the observed values of the animal characteristics tested. Values in bold indicate how much the weighted characteristics influenced the model that determined the dominance value.

The dominance value was correlated with animal characteristics that had an influence on the statistical model (Table 12-1). The presence of horn, a unique characteristic of intermediate and subordinate animals, did not influence our models, but it was still correlated with the dominance value.

Table 12-1 Spearman's test to correlate each main animal characteristic with the dominance value.

Animal characteristics	Dominance value	
	Correlation coefficient	p-value
Age	0.55	0.0028
Body mass	0.67	0.0001
Withers height	0.68	0.0001
Body length	0.62	0.0005
Horn presence	-0.54	0.0038

Animal characteristics with p<0.05 are significantly correlated by the Spearman's test.

Presence of the horn influenced (p=0.034) the dominance value of intermediate and subordinate animals. Differences were found (p<0.001) in horn circumference (I - 14.75cm; S - 11.70cm) and horn length (I - 21.13cm; S - 15.10cm) between these categories. However, no difference was found (p=0.944) in the distance between horns (I - 25.00cm: S - 23.40cm). Circumference (r=0.85; p<0.05) and horns length (r=0.82; p<0.05) are more correlated with age than the distance between the horns (r=0.44; p<0.05).

Social category influenced (p<0.05) the frequency of dominant, intermediate, and subordinate remaining at the feeder. Dominant (84.3%) and intermediate (75.2%) animals spend more time (p<0.05) at the feeder than the subordinate (59.5%); however dominant animals tended (p=0.093) to spend more time at the feeder than the intermediate animals. There was no difference (p=0.89) in the time at the feeder between horned and non-horned cows from lower social categories (intermediate and subordinate).

12.2 DYADIC-LEVEL

The stronger role of age in relation to body mass (Figure 12-2) was also confirmed (p<0.001) by dyadic level analysis. Thus, the probability of an older cow being dominant in the pair was higher than that of a younger cow (Fig. 3). From 351 dyads, the focal cow was dominant in 204, and the main combinations found were: older + heavier (81 dyads), younger + heavier (45 dyads), and older + lighter (33 dyads). The others combinations correspond to same age + heavier (21 dyads), younger + lighter (10 dyads), same age + lighter (8 dyads), older + same weight (4 dyads), younger + same weight (1 dyad), and same age + same weight (1 dyad). The focal cow was subordinate in 140 dyads from the total (351). The main combinations found to subordinate focal cow were: younger + lighter (72 dyads), older + lighter (30 dyads), and same age + lighter (22 dyads). The others combinations correspond to younger + heavier (10 dyads), older + heavier (3 dyads), same age + heavier (1 dyad), younger + same weight (1 dyad), and same age + same weight (1 dyad). From the total (351 dyads), the less frequency of combination (7 dyads) found was when the cows had the same social position; being them: older + lighter (5 dyads), same age + heavier (1 dyad), and younger + older (1 dyad).

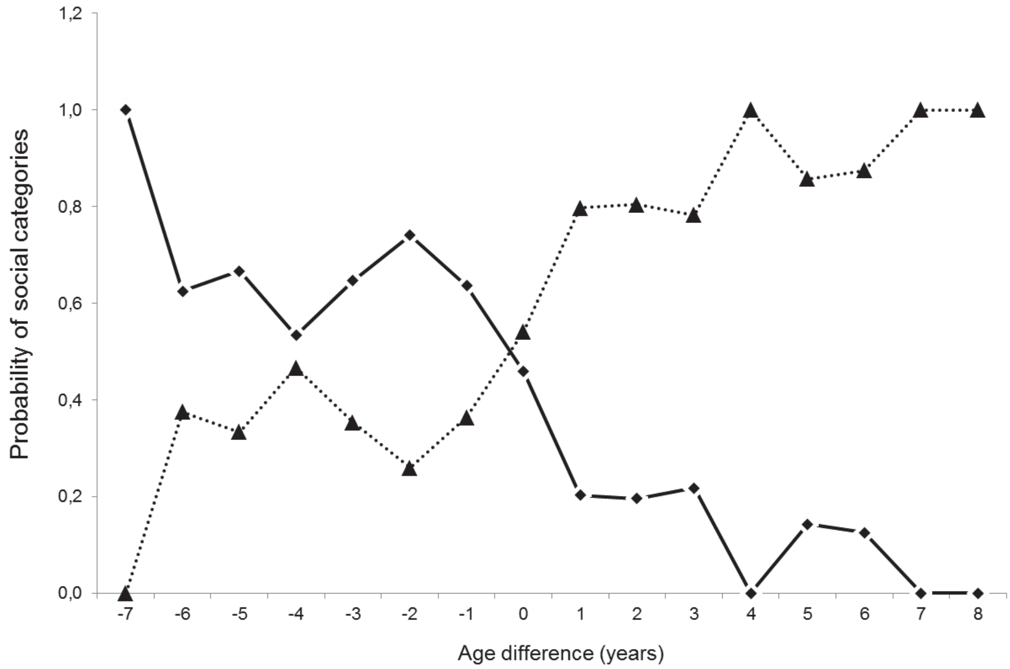

Figure 12-2 Probability of being a dominant or subordinate animal in relation to the age difference between the focal animal and its opponent (Dominant - triangles and dash-dotted line; Subordinate - rhombus and solid line).

Quantitative difference in body mass also had an influence ($p<0.001$) on the models; the probability of being dominant in the pair increased with the larger body mass advantage of the focal cow in a pair (Figure 12-3). When categorized by difference in body mass, the percentage of dyads in which the lightest, but oldest, cow was dominant was 71.8% when she had a small disadvantage (between 1 and 50 kg) and 28.2% when she had a moderate disadvantage (between 51 and 100 kg). Meanwhile, no older and lighter cow was dominant with an extreme disadvantage (> 100 kg).

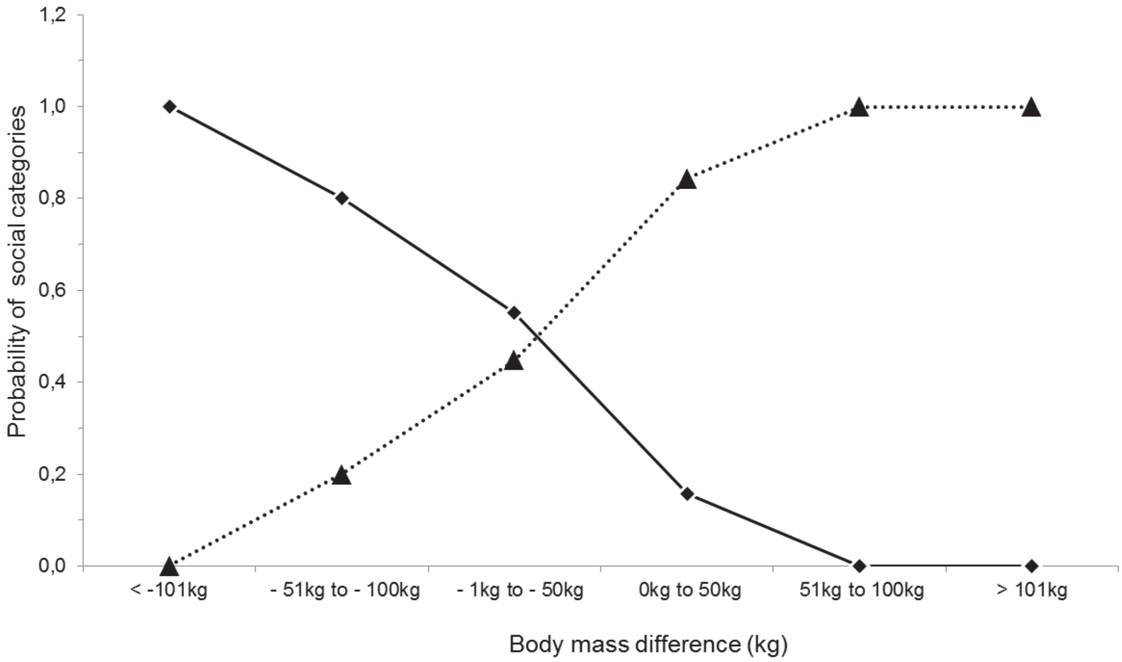

Figure 12-3 Probability of being a dominant or subordinate in relation to the body mass difference between the focal animal and its opponent (Dominant - triangles and dash-dotted line; Subordinate - rhombus and solid line).

When evaluating the horned cows as a focal animal, they were dominant only in 15 dyads, out of which in 10 dyads the focal animal was the heaviest, but not always the oldest (D older = 3; D younger = 4 and D same age = 3); in the other, 5 dyads the focal animal was the lightest and both animals at the pair had the same age. Nevertheless, in all dyads (44 dyads) in which the horned animal was subordinate, they were the younger and lighter.

13 DISCUSSION

In a mixed herd, our study shows that age and body mass were decisive factors in determining social dominance at both herd and dyad level for horned and non-horned cows. In addition, presence of horns was a unique characteristic of intermediate and subordinate animals, considering that these animals were the youngest and lightest in the herd; therefore, they did not use the horn to gain an advantage over the others (group and dyads). The presence of horns is a characteristic that seems to be more important in determining the social position of individuals in newly formed groups, as they offer an advantage in aggressive interactions (Bouissou

1972). Even though the horn can be used as a weapon, age is the main factor in stable groups for determining social status (Šárová et al. 2013) since horned cows were only reported as dominant in herds where they were more likely to be older than most animals (Beilharz and Zeeb 1982).

Positive, significant correlation among dominance value, age, and body mass was verified when we analyzed both herd and dyad level; this same pattern was also found in other studies (Šárová et al. 2013; Hussein et al. 2016). When analyzed social dominance of grazing dairy cows in a level group, Hussein et al. (2016) found a positive correlation of the dominance value with age, weight and milk production. Meanwhile, Šárová et al. (2013) found that age superiority had a stronger influence on the direction of social dominance in pairs than body mass superiority, both herd and dyad level. The dominant-subordinate relationship established between two animals is very stable over the years, and it is rarely reversed (Barroso et al. 2000; Šárová et al. 2013). However, when the dominance relationship is reversed, usually the youngest animal has reached its adult body mass, thus challenging older, but lighter, animals to which they were previously subordinate (Favre et al. 2008).

The probability that a focal animal will be dominant in a dyad increased as its quantitative difference in age and body mass became greater than its opponent. Previous studies assessing social status in ungulate females suggest that dominance increases with age, perhaps because dyadic relationships are established early life when the older animal is generally larger than the younger animal (Barroso et al. 2000 in goats; Favre et al. 2008 in ewes; Šárová et al. 2013 in beef cows). Our study supports this information since body length was also a physical characteristic that influenced the dominance value of the individual. Although intermediate animals showed greater horn circumference and length, these characteristics are correlated with age. Horns never stop growing (Tidière et al. 2017), so they have a significant correlation with the age of the animals, as found in this study. However, within the species, there is a great variation in the length and circumference of the horn between individuals (Lundrigan 1996; Preston et al. 2003; Tidière et al. 2017).

At no time during our observations did a victim suffer injury from a horned cow. The chance of injury in animals is more related to management and facility practices on the farm (Menke et al. 1999) than animal aggression. Moreover, a management practice adopted at this particular farm involves the periodic trimming of horn tips, reducing the chance of injuries. Thus, it is important to emphasize that horned dairy cows can be kept in the herd, but the farms must adapt the management and facilities according to the animal’s breed (Menke et al. 1999, 2015). As an example, one facility adaptation is the introduction of structural elements (such as Y-shaped barriers) into the free resting area in loose-housing systems. This adaptation has the potential to improve the welfare of horned cows by reducing aggressive interaction (Menke et al. 2015).

We verified that the adopted dimension (1 m linear/ animal) in this study was sufficient for all animals to have simultaneous access to the feeder. This may be related to the fact that we have evaluated a breed that has small horns. When considering the size of the horn, it is possible that the linear space at the feeder should be larger to allow another cow to approach the feeder safely. However, there is no consensus on the ideal feeder space in the literature and these studies evaluated non-horned animals (DeVries et al. 2004; Rioja-Lang et al. 2012; Greter et al. 2013; Hetti Arachchige et al. 2014). Yet, we strongly suggest that other studies should be conducted to measure facilities for different breeds that have larger horns than those found in this study (16.8 cm horn length).

To assess the influence of phenotypic characteristics on the animals' dominance value, we chose to observer the time they were at the feeder. Despite having enough space for all animals to access the feeder and an abundant amount of silage, the fact of offering a supplement in line, makes it a competitive situation, as the feeder had no physical barrier. Absence of physical barrier further increase competition (Huzzey et al. 2006; Hetti Arachchige et al. 2014). Another determining factor to consider the feeding area as a competitive situation was the stocking density, whereas in the pasture the cows had a disposal 74m²/ cow, in the feeding area they had only 11m²/ cow. It is known that high-ranking animals are more likely displace low-ranking when in competitive situations (Thouless 1990; Lea et al. 2014); however, when supplementation is provided in a pasture area, fewer aggressive interactions occur, as animals of low-ranking animals seek other areas to feed, avoiding aggressive interaction (Bica et al. 2019b).

In a mixed herd, contrary to what was expected, non-horned cows remain longer at the feeder, as they were dominant. Nevertheless, the lower frequency of subordinate animals at the feeder does not mean that they ate less food. In an attempt to ensure their nutritional status, subordinate animals adopt strategies to facilitate getting to food. As an example, these animals can increase the rate of food intake

(Shrader et al. 2007; Fiol et al. 2019), or even graze, while the dominant animals are ruminating or ingesting supplement (Bica et al. 2019b). Thus, these animals can ingest food in amounts similar to those of dominant animals (Beauchamp 2006; Fiol et al. 2019).

Popular pressure for production systems that respect and guarantee animal welfare is increasingly strong. Therefore, we have brought here a situation that dairy farms will have to deal with in the coming years. As we have shown, there are other characteristics more important than the horn for determining the animal's social position, and the mere fact of dehorning them will not diminish disputes over resources. Thus, this observational study brings an important situation on the scenario of organic farms, it is possible to keep the cows in their natural state (with horns), however, it is necessary to adopt some precautions.

14 CONCLUSION

We found that the social position of an animal was influenced by its age, body mass, and body length. Presence of horn was a unique characteristic of intermediate and subordinate animals and they did not use this feature to gain an advantage in determining their social position. Social position influenced frequency at the feeder, as the dominant animals (older and heavier) remained longer at the feeder. Even in the lower-ranking categories, horned animals did not remain at the feeder any longer compared to non-horned animals.

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Conflicts of interest

The authors declare no conflicts of interest.

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16 CHAPTER III - DEVELOPMENT AND APPLICATION OF AN AUTONOMOUS DATA LOGGER TO MEASURE ENVIRONMENTAL VARIABLES IN LIVESTOCK FARMING

Matheus Deniz[1*], Karolini Tenffen de Sousa[1], Isabelle Cordova Gomes[1], João Alberto Fabro[2], Marcos Martinez do Vale[1], João Ricardo Dittrich[1]

[1]Laboratório de Inovações Tecnológicas em Zootecnia (LITEZ), Departamento de Zootecnia, Universidade Federal do Paraná (UFPR), Curitiba – PR, Brazil.

[2]Laboratório Avançado de Sistemas Embarcados e Robótica (LASER), Universidade Tecnológica Federal do Paraná (UTFPR), Curitiba – PR, Brazil.

*Corresponding author:
Matheus Deniz; ORCID: 0000-0001-8079-0070
E-mail: matheus-utfpr@hotmail.com;

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Abstract

The environmental conditions of livestock farming exhibit a wide degree of variability. In this context, we developed the ADEF, an autonomous datalogger to better understand the degree of environmental variables that farm animals are exposed. Each ADEF consists of a set of components: microcontroller, memory card, real-time clock module, ambient sensor (DHT22), two thermal sensors (DS18B20), and an external battery. To validate the accuracy of ADEF, two stages were performed: (1) evaluation in a controlled environment; and (2) evaluation in the field. In both validation, uncertainty analyses were performed in order to determine if a bias correction would be necessary. In the controlled environment, the ADEF recorded consistent data associated with low measurement uncertainty. The high and significant coefficient of determination (~0.9; $p<0.05$) between the ADEF and commercial datalogger indicated statistical model quality and confirmed the accuracy of the measured data. In the field, a total of 40,100 measurements were used for subsequent analysis. Furthermore, the hourly variation in the ADEF variables showed the same pattern and a high correlation (~0.9) with the data from the nearest meteorological station. In the field, environmental variables measured by the ADEF demonstrated low hourly dispersion associated with low relative standard uncertainty. The performance of the ADEF system was satisfactory both controlled environment and field, demonstrating that the ADEF can be easily applied as a low-cost tool that allows a more efficient approach to measure the environmental variables in the field.

Keywords: Big data. Low-cost devices. Microcontroller. Precision livestock farming. Thermal environment.

17 INTRODUCTION

The environment is a determining factor for livestock, as it influences the productivity of the farm (Renaudeau et al. 2011, 2012) and the quality life of the animals (Shock et al. 2016). Due to climate change, the effects of the thermal environment will be increasingly intense, mainly due to increases in air temperature and the frequency of extreme weather events (Nidumolu et al. 2014); this could affect the availability of grain and pasture, and also the presence of pests and parasites (Gauly et al. 2013). This situation has promoted negative effects on production (Bohmanova et al. 2007; Hammami et al. 2013), fertility (Hansen 2009), and animal health (Sanker et al. 2013), in addition to increasing the risk of mortality (cows: Vitali et al. 2009; laying hens: Riquena et al. 2019). Thus, production systems that improve quality of life for farm animals are gaining attention in the scientific community (e.g., the reviews of Kadzere et al. 2002; Das et al. 2016; Dash et al. 2016; Polsky and von Keyserlingk 2017; Herbut et al. 2019).

The thermal environment is composed of air temperature, relative humidity, and solar radiation; and the intensity of these factors can cause thermal stress in farm animals. Thermal stress occurs when animals experience conditions outside their thermal comfort zone (Kadzere et al. 2002) and are unable to dissipate (or receive/produce) enough heat to maintain thermal balance. The thermal environment can affect animal performance immediately or have a delayed impact (St-Pierr4e et al. 2003; Herbut et al. 2018). Thus, better understanding of how animals respond to climate factors is essential for livestock farming to be able to adapt new challenges (Hill and Wall 2014). However, the influence of the thermal environment on animals is rarely quantified on farms, resulting in a lack of precise control of the environment for the livestock. To address this lack of control, advanced techniques and precise equipment are needed, which record environmental data and allow us to assess their effects on livestock (Laberge and Rousseau 2017).

Based on the principles of precision livestock farming (autonomous, precise, and continuous), technological advances, associated with the low-cost of electronic components (e.g. sensors, smart cameras, and microphones, etc.) have resulted in the emergence of integrated physical devices (Neethirajan et al. 2017). These devices are able to provide support to accurately monitor and manage production systems (Neethirajan et al. 2017); thus, the problem can be identified on the farm before it leads to a condition of animal stress (King 2017). In this context, to apply precision livestock farming, it is necessary to develop a local environmental control system integrating automated measurements using precise sensors, making it possible to infer about biological and physical processes with data from the local environment (Laberge and Rousseau 2017; Hoffmann et al. 2019).

A set of multiple sensors in a single equipment allows robust and practical measurement of environmental variables for application in livestock farming. Thus, our study set out to: first to develop an autonomous datalogger to measure environmental variables (denominated the ADEF) and validated with data collected in a control environment compared with a reference system; secondly, we applied and evaluate the performed of ADEF to collect data in a livestock farm between March and August 2020; and finally, we determined the standard uncertainty associated with the ADEF measurements from the both collections.

18 MATERIALS AND METHODS

In the current study, we use low-cost components with control based on a microcontroller, programmed by open-source software, to develop five autonomous datalogger named ADEF, which simultaneously measures environmental variables. Each ADEF consists of a set of components: (1) microcontroller (Arduino® Nano), (2) module of removable flash memory card (e.g., micro SD), (3) Real-Time Clock module (RTC - DS1307), (4) ambient sensor (DHT22), (5) two thermal sensors (DS18B20), and (6) external battery (Figure 18-1).

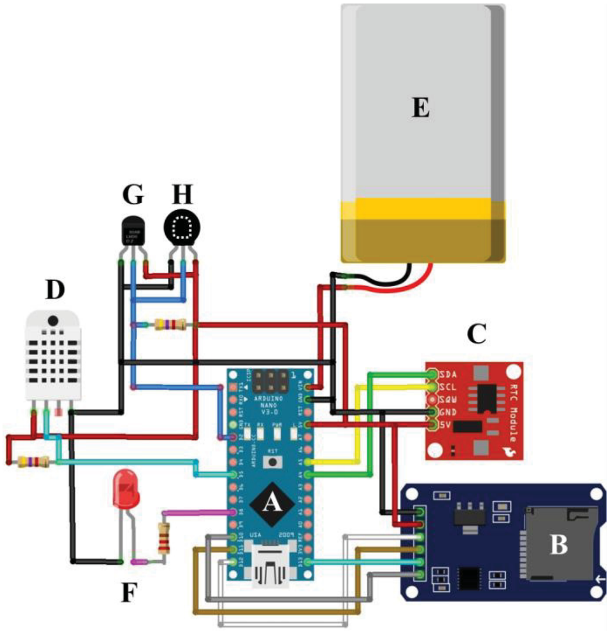

Figure 18-1 Schematic representation of the autonomous datalogger to measure environmental variables board interface: (A) microcontroller, (B) micro SD module, (C) RTC module, (D) ambient sensor (DHT22), (E) external battery, (F) operating light, (G) thermal sensor , and (H) thermal sensor 2.

The ambient sensor (DHT22) provides instantaneous values of air temperature (-40º to + 80ºC scale, ± 0.5°C precision; and 0.1°C resolution) and relative humidity (0 to 100% scale ± 2.0% precision; 0.1% resolution). Each ADEF has two thermal sensors (-55ºC to 125ºC scale ± 0.5 ° C precision; and 0.1°C resolution), that can provide the temperature of the interest; for example, surface temperature and black globe temperature (BGT). However, to measure the BGT is necessary to fix the thermal sensor at the center of a hollow black sphere (Ramirez et al. 2018; Vega et al. 2020). The information of date and time was provided by the RTC module, which operates with its own lithium battery. This ensures that data are preserved even without external power. The RTC contains a circuit which is able to detect power failures, automatically activating its own battery to avoid data loss. The power supply to the ADEF circuit was provided by a lithium battery (5V, 9000mAh). The data obtained by all electronic components were processed by the microcontroller and the results were stored in a micro SD card.

The ADEF circuit (microcontroller, RTC module, micro SD module, and battery) was housed in a 10cm x 7.3cm x 4.1cm (length x width x depth) weatherproof box. Three cable grips were installed to provide watertight connections for the components (DHT22 and DS18B20). The components of the ADEF have 1.5m long cables, which allow versatility, as the device can be installed at different heights and in different production scenarios (Figure 18-2).

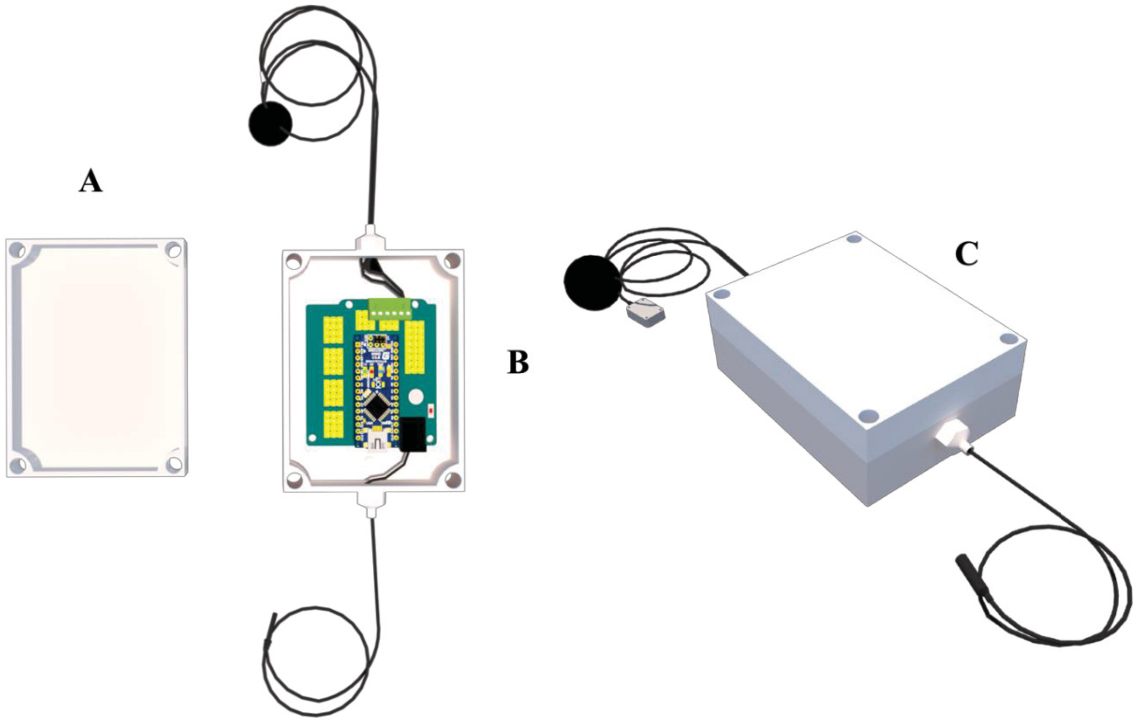

Figure 18-2 Schematic representation of the ADEF weatherproof box and cable grips: (A) box cover, (B) inside view of the box with ADEF circuit, and (C) fully assembled ADEF data acquisition unit.

18.1 OPEN SOFTWARE

The firmware was developed in the Processing language for Arduino (a simplified version of C/C++ programming languages). The Integrated Development Environment (Arduino IDE) was used to compile and upload the program to the ADEF. The developed code activates the peripheral circuits (electronic components), such as reading the values measured by each component, monitoring the battery level, controlling the digital and analog data reception systems, and sending the data to the storage unit (micro SD), as shown in the block diagram in Figure 18-3.

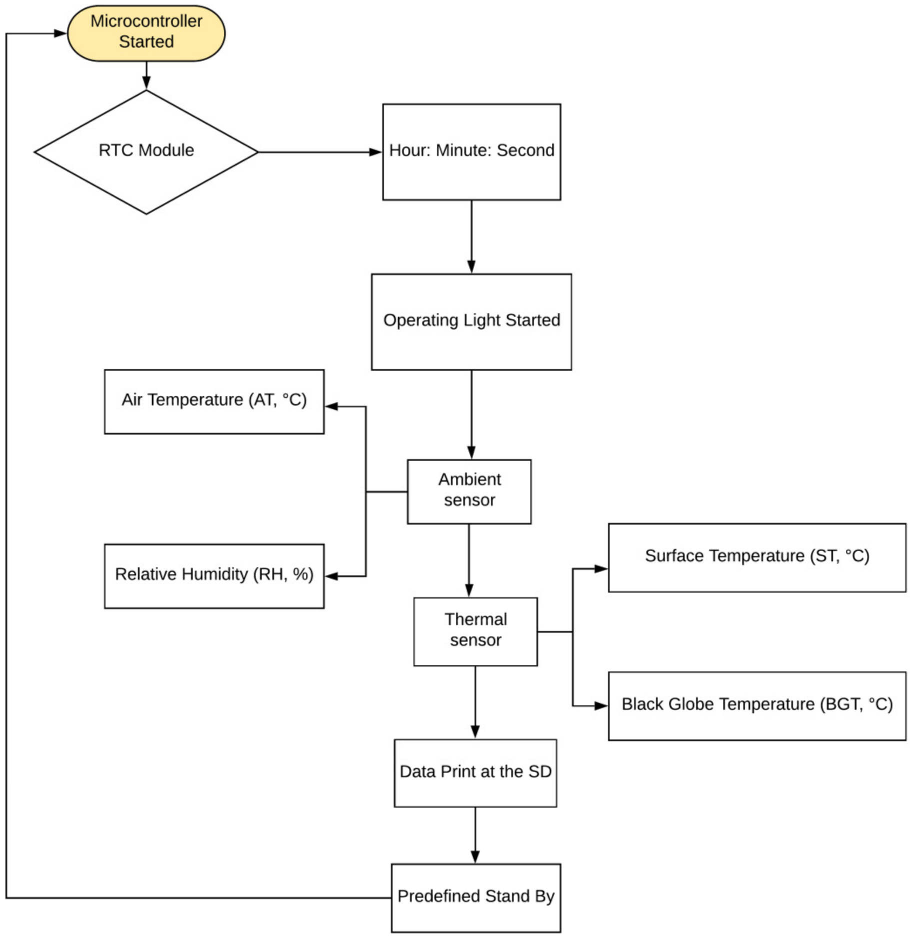

Figure 18-3 Block diagram of the autonomous datalogger to measure environmental variables (ADEF).

18.2 EVALUATION OF ADEF MEASUREMENTS

To validate the accuracy of five ADEFs, two stages were performed: (1) evaluation in a controlled environment; and (2) evaluation in the field. In addition, the battery lifetime of the ADEF was estimated with the current specifications of each electronic component (Table 18-1), time duration in working state, and time duration in standby state.

Table 18-1 Summary of currents in both working state and standby state for each component.

Description	Current in Working state (mA)	Current in Standby state (mA)
Microcontroller	280	1.2
SD module	80	0.2
RTC module	1.5	0.2
Ambient sensor	2.5	0.13
Thermal sensors[a]	1.5	0.001

[a]ADEF has two thermal sensors.

Time averaged power consumption was estimated by equation 1, proposed by Ngo et al. (2020). For an electronic component i^{th}, the $I_i^{working}$ and the $T_i^{working}$ are the current and the time spent in a working state, respectively. While $I_i^{standby}$ and $T_i^{standby}$ are the current and time consumed in a standby state of all components (n). Thus, I_{av} represents the time-averaged power consumption in relation to the total time of period duration.

$$I_{av} = \frac{\sum_n I_i^{working} \, T_i^{standby}}{T_{period}} \quad (1)$$

Where: I_{av} is time-averaged power consumption (mA), and T_{period} is total time of period duration. Total time of period duration (T_{period}) was calculated by equation 2.

$$T_{period} = \sum_n T_i^{working} + \sum_n T_i^{standby} \quad (2)$$

The total battery lifetime (T_{work}) was calculated by equation 3.

$$T_{work} = \frac{B}{I_{av}} \quad (3)$$

Where: B is battery capacity (mAh), and I_{av} is time-averaged power consumption (mA).

18.2.1 Stage 1 - Evaluation in a controlled environment

This stage was carried out at the “Laboratório de Inovações Tecnológicas em Zootecnia” of the “Universidade Federal do Paraná”. The stage 1 was divided in two steps: step 1 - evaluation of the thermal sensor (DS18B20) accuracy; and step 2 - evaluation of the ambient sensor (DHT22) accuracy. In both steps, the data from the five ADEFs were compared with data obtained from five commercial data logger (EL-USB-2 Lascar®; air temperature: -35º to + 80ºC scale, ± 0.5°C precision; and 0.1°C resolution; relative humidity: 0 to 100% scale, ± 2.25% precision; 0.5% resolution).

In the step 1, the thermal sensors (n = 10) of ADEFs and the commercial data loggers were placed in a forced-ventilation oven (model MA033/1080, Marconi, Brazil) with a controlled temperature (52°C ± 0.8) for 48 hours. In the step 2, the ADEF and the commercial datalogger were placed in a room with the temperature controlled by air conditioning (20°C ± 5) for 12 hours. In both steps, the ADEF and the commercial datalogger were programmed to record data every 1 minute. During all time of both steps, we guarantee that they were kept closed. Also, the forced-ventilation oven is shielded, what allows good insulation; and the room is coated with extruded polystyrene plates (5mm) to guarantee good insulation.

18.2.2 Stage 2 – Evaluation in the field

As part of a larger study, a total of five ADEFs were placed in the pasture area at a commercial dairy farm. Data collection was carried out between March and August 2020, on four consecutive days per month. The selection of the experimental days was based on multiple weather forecasts and had the same climatic characteristics (without rain and low cloudiness).

18.2.2.1 Farm location and climate pattern

This stage was carried out at the “Estação de Pesquisa Agroecológica - CPRA” at the “Instituto de Desenvolvimento Rural do Paraná”, Paraná state, in Southern Brazil (25°26'41"S, 49°11'33" W). According to the Köppen classification, the climate of the region is characterized as wet maritime temperate (Cfb), with a minimum temperature below 18°C (79% relative humidity) and a maximum temperature above 22°C (82%

relative humidity), with an average monthly rainfall above 70mm (INMET 2009; Alvares et al. 2013).

18.2.2.2 ADEF sensor position

Each ADEF was fixed to a tripod (Figure 18-4). At this stage we used one thermal sensor to measure soil surface temperature and other to measure black globe temperature. For measure the soil surface temperature, the thermal sensor was located in the soil below the pasture (de Sousa et al. 2021a). For measure the black globe temperature, a thermal sensor was fixed at the center of a hollow black sphere (de Sousa et al. 2021a). In this study, we did not validate the use of thermal sensor DS18B20 to measure the black globe temperature (BGT) because it was already performed by Vega et al. (2020), which when evaluating the performance of different sensors to measure BGT, recommended the use of DS18B20 sensor. However, we emphasized that the accuracy of the thermal sensor of the ADEF was tested in the first stage of this study. The BGT and the ambient sensor were located 1.3m above the soil. The ambient sensor was located inside a meteorological shelter (ISO 7726, 1998; Barbosa et al. 2008), obtained through 3D printing using a polylactic acid filament.

Figure 18-4 Sensors positioned for data collection during the evaluation in the field.

18.2.2.3 Environmental variables

For the environmental variables measure, five ADEFs were positioned equidistantly inside of a paddock (Figure 18-5) between the side fences (17.5 m). The environmental variables of air temperature (AT, °C), relative humidity (RH, %), black globe temperature (BGT, °C), and soil surface temperature (SST, °C) were recorded for seven consecutive hours (8:00 to 14:55) every 5 min., and averages were generated every 1 hour (e.g., 8:00 to 8:55). Although, our study did not involve data collection with animals; we highlight that the objective of this study was evaluate the performance of ADEF in measure environmental variables on a commercial dairy farm (CPRA). Thus, the period choice was based on the interval between milking adopted by CPRA. In addition, the period (8:00 to 14:55) is related to the times of the maximum meteorological variables (INMET 2009), that can lead to dairy cows' thermal stress (de Sousa et al. 2021a).

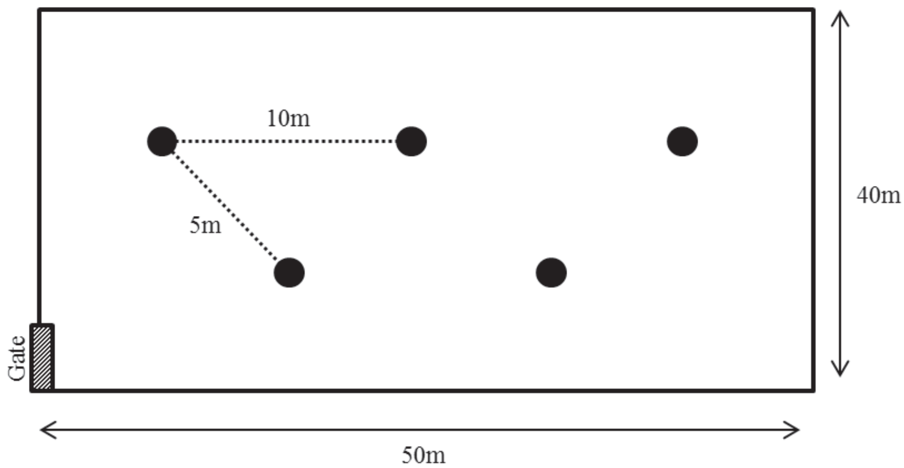

Figure 18-5 Schematic representation of the distribution of the five ADEFs in the paddock.

18.3 STATISTICAL ANALYSIS

In summary, all analyses [descriptive (average, minimum, maximum, and confidence intervals) and confirmatory] were performed using Statistical Software R version 3.5.3 (R Core Team 2019). In order to confirm the fitness of the models, we inspecting the residual in the graphs associated with the direct likelihood approach (Lindsey, 1998). The models were adjusted through the maximum likelihood-Laplace approximation method in the statistical package lme4 (Bates et al. 2015). The confidence intervals were estimated using Type II Wald chi-square tests and the fit of the models was given by a likelihood-test. Therefore, the model good fit was considered when the residues showed constant variance and were randomly distributed around zero.

18.3.1 Details of the statistical analysis

Initially, descriptive statistics were used to view the raw data from the ADEF and commercial datalogger. The initial tests showed that the data from the ADEFs were not identical; therefore, it was necessary to investigate whether this variation would influence the validation of the equipment. The non-parametric Wilcoxon t-test was applied to the paired data (measured at the same time), for the two thermal sensors in a single ADEF, while the Wilcoxon-Mann-Whitney non-parametric test was used to assess whether there was a significant difference among the ADEFs, both at a 5% significance level. A simple linear regression with a 95% confidence level was used to estimate the coefficient of determination (R^2) between the data from the ADEF and from the commercial datalogger. Finally, to compare the data between the ADEF and commercial datalogger, multilevel analysis by Generalized Linear Models (GLM) was used, with a 95% confidence level. The ADEF calibration was performed through readings in parallel with the commercial datalogger with the aid of the maximum likelihood-Laplace approximation method, which made it possible to establish a sequence of ordered pairs aiming at curve fitting techniques. Each variable was analyzed separately and for the GLM models, Gamma distribution with the logarithmic link function was used.

In the field evaluation, to analyze the relationship between the data of ADEF (relative humidity and air temperature) with that recorded by the nearest meteorological station, a Spearman correlation with a confidence interval of 95% was used. During the experimental period, we randomly chose four days and tested of degree, and direction of the relationship between the variables. The meteorological station (station ID - 25254905) belong to the SIMEPAR (in Portuguese – “Sistema de Tecnologia e Monitoramento Ambiental do Paraná”) was located in Piraquara - PR (15km distance from the experimental area).

18.3.2 Details of analytical and uncertainty analysis

Uncertainty assessments (types A and B) were performed for each ADEF component (Gao et al. 2016; Ramirez et al. 2018). The standard uncertainty associated with a measurement is a statistical approximation of the measurement error obtained from the propagation of the main sources of uncertainty (Taylor and Kuyatt 1994; JCGM 2008). The results of the uncertainty budget were propagated through analytical solutions (based on Taylor and Kuyatt 1994; JCGM 2008; Gao et al. 2016; Ramirez et al. 2018) that use measurements as inputs, to finally determine the combined uncertainty associated with the calculated value. To determine the uncertainty of each component, the information (Table 18-2) provided by the manufacturers (datasheet) was used as contributors to the equation, and the values obtained by the commercial datalogger were used as a reference variable (in the field stage, an average value for each 1h was determined as the reference value). We assumed a rectangular probability distribution (JCGM 2008) for the parameters for which no information is provided regarding the source of the values; therefore, values are assumed to have an equal probability of existing within the stated range.

Table 18-2 Uncertainty budget summary provided by the manufacturers used as equation contributors to determine the standard uncertainty associated with ADEF measurements.

Parameter	Description	Sensor	Parameters	Standard uncertainty[a]	Unity	Probability distribution
AT	Air temperature	DHT 22	Repeatability	0.2	°C	Normal
			Accuracy	0.5	°C	Rectangular
			Standard resolution	0.1	°C	Rectangular
			Stability over 2 years	±2	%	Rectangular
RH	Relative humidity	DHT 22	Repeatability	1	% RH	Normal
			Accuracy	2	% RH	Rectangular
			Standard resolution	0.1	% RH	Rectangular
			Stability over 2 years	±2	%	Rectangular
Thermal sensor	Temperature	DS18b20	Repeatability	0.2	°C	Normal
			Accuracy	0.5	°C	Rectangular
			Standard resolution	0.1	°C	Rectangular
			Stability over 2 years	±2	%	Rectangular

[a]manufacturer specifications

All analytical solutions were performed based on Taylor and Kuyatt 1994; JCGM 2008; Gao et al. 2016; Ramirez et al. 2018. We highlight that is possible to apply the equations for all components. However, so that errors not occurred, it is necessary to use the reference value corresponding to the component that is seeking to determine the uncertainty. Based on this, we described the uncertainty associated with the unit of measure (°C or %RH) of each component based on Ramirez et al. (2018). Where the unit "°C" corresponds to the temperature measured by the ambient sensor (DHT22) and the thermal sensor (DS18B20); while the unit "%RH" corresponds to the relative humidity measured by the ambient sensor (DHT22).

The standard uncertainty for a sample X of ADEF (equation 4) associated with the reference measurement of the commercial datalogger was determined by the propagation of the source error (temperature, °C and relative humidity, %) through the analytical solution derived from the component error and sample deviation.

$$\Delta_{ADEF} = \overline{x} \pm \sigma_x \tag{4}$$

Where: $\Delta_{ADEF}$is the standard uncertainty, $\overline{x}$ is the average of a sample of measures, and σ_x is the deviation of the sample. The σ_x was calculated by the equation 5.

$$\sigma_x = \sqrt{\frac{1}{(n-1)} \sum (\bar{x} - x_n)^2} \tag{5}$$

Where: σ_x is the deviation of the sample, n is the number of measurements, $\overline{x}$ is the average of a sample of measures, and x_n is a value measured.

The tendency of propagating the standard uncertainty for a sample x of the ADEF associated with the reference measurement of the commercial datalogger was determined by equation (6).

$$\beta = \overline{x}_{ADEF} - \overline{x}_{ref} \tag{6}$$

Where: β is the trend of propagating the standard uncertainty, $\overline{x}_{ADEF}$ is the average of ADEF values (°C or %RH), and $\overline{x}_{ref}$ is the average of reference values from the commercial datalogger (°C or %RH).

The combined standard uncertainty of the ADEF (equation 7) was determined as the root-sum square of the standard uncertainty (types A and B) associated with each sample of the electronic components.

$$u_{ADEF} = \sqrt{\left(u(\overline{y})\right)^2 + \left(u(\overline{z})\right)^2 \ldots} \tag{7}$$

Where: $u_{ADEF}$is the combined standard uncertainty of ADEF (°C or %RH), $u(\overline{y})$ is type A uncertainty (°C or %RH), and $u(\overline{z})$ is type B uncertainty (°C or %RH).

The standard uncertainty of type A (uncertainty assessment method by statistical analysis of a series of observations) was determined by equation (8) and applied to parameters with the probability of normal distribution.

$$u(\overline{y}) = \frac{S}{\sqrt{}_n} \tag{8}$$

Where: $u(\overline{y})$ is type A uncertainty (°C or %RH), S is the experimental standard deviation, and n is number of measurements (n is independent observations).

The standard uncertainty of type B (non-statistical uncertainty assessment method; obtained from the manufacturer's information) was determined by equation (9), and applied to the parameters with the probability of rectangular distribution.

$$u(\overline{z}) = \frac{\alpha}{2 * \sqrt{}_3} \tag{9}$$

Where: $u(\overline{z})$ is type B uncertainty (°C or %RH), and α is standard uncertainty provided by manufacturers (°C or %RH).

To define whether ADEF was acceptable (e.g. it had some significant bias) we determined the expanded uncertainty (equation 10).

$$U = k * u_{ADEF} \tag{10}$$

Where: U is the expanded uncertainty, k is the coverage factor determined based on the level of confidence required for the interval range y - U to y + U, and u_{ADEF} is the combined standard uncertainty (°C or % RH).

To define the value of k (equation 11) it was necessary to determine an effective number of degrees of freedom.

$$v_{eff} = \frac{{u_{ADEF}}^{4}}{\left(\frac{u(\overline{y})}{n-1}\right)} \qquad (11)$$

Where: v_{eff} is the coverage factor, u_{ADEF} is the combined standard uncertainty (°C or % RH), $u(\overline{y})$ is type A uncertainty (°C or % RH), and n is number of measurements (n independent observations).

The relative uncertainty (Ω) (quotient between the expanded uncertainty (U) and the most probable value of the quantity) was determined by equation (12). The relative uncertainty indicates the accuracy of the measurement performed (JCGM 2008), and the lower the relative uncertainty, the greater the degree of accuracy.

$$\Omega = \frac{U}{\overline{x}_{ADEF}} \qquad (12)$$

Where: Ω is relative uncertainty, U is expanded uncertainty, and $\overline{x}_{ADEF}$ is the average ADEF value (°C or % RH).

19 RESULTS

19.1 BATTERY LIFE TIME OF ADEF

The time-averaged power consumption (I_{av}) was approximately 3.16 mA, with a mean time duration (T_{period}) of 200 ms. Thus, the expected battery lifetime is roughly 2,800 h, or approximately 118 days if the proposed datalogger runs 24 h per day.

19.2 STAGE 1 – EVALUATION IN A CONTROLLED ENVIRONMENT

19.2.1 Step 1 – Evaluation of thermal sensor accuracy

In the evaluation of the thermal sensor accuracy (48 hours), 28,880 data were measured. Both dataloggers (ADEF and commercial) had similar mean values of temperature (ADEF = 52.60°C and commercial = 52.30°C). The ADEF recorded more consistent data (Figure 19-1) and with a lower variation that the commercial device (ADEF - range: 52.20°C to 52.83°C and commercial datalogger - range: 50.57°C to 52.50°C).

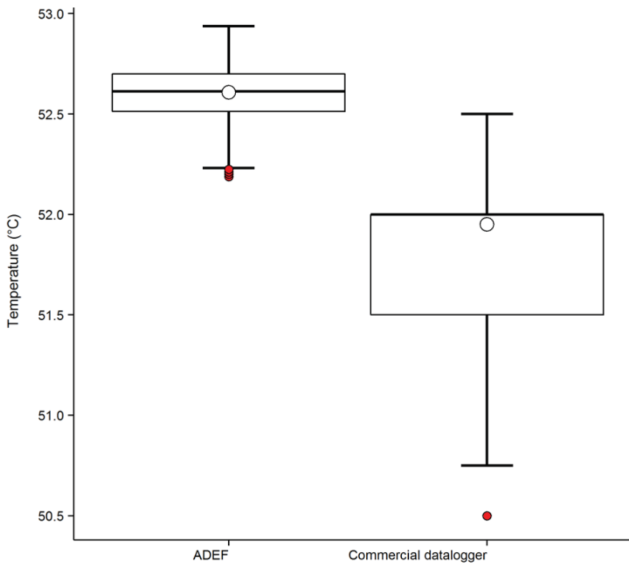

Figure 19-1 Box-plot diagram of temperature (°C) measured by both dataloggers (ADEF and commercial) in a forced-ventilation oven.

There was no difference ($p = 0.72$) of data recorded between the thermal sensors of each ADEF according to the Wilcoxon t-test. Although the numerical values recorded by all thermal sensors ($n = 10$) of the five ADEFs were not exactly equal, there was no difference ($p = 0.81$), and they did not differ in distribution, according to the Wilcoxon-Mann-Whitney test. Furthermore, there was no difference ($p = 0.89$) between the ADEF and the commercial datalogger for the temperature measured in a forced-ventilation oven. The hourly summarized data showed a high coefficient of determination ($R^2 = 0.989$), i.e., 98.9% of the ADEF variation can be explained by measurements from the commercial datalogger (Figure 19-2).

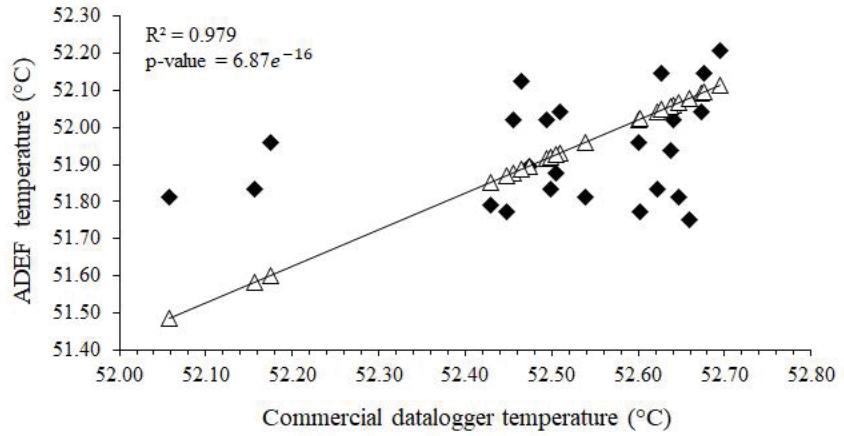

Figure 19-2 Temperature distribution (°C) measured in a forced-ventilation oven by both dataloggers (ADEF = white triangles and commercial datalogger = black rhombuses).

19.2.2 Step 2 – Evaluation of ambient sensor accuracy

In the evaluation of the ambient sensor accuracy (12 hours), 3,600 data were measured. There were no differences in the relative humidity ($p = 0.189$) and air temperature ($p = 0.168$) between the dataloggers (ADEF and commercial). The data measured by both dataloggers showed a high coefficient of determination (Figure 19-3). There was significance in the determination coefficient for variables (relative humidity and air temperature), indicating model quality and confirming the data accuracy.

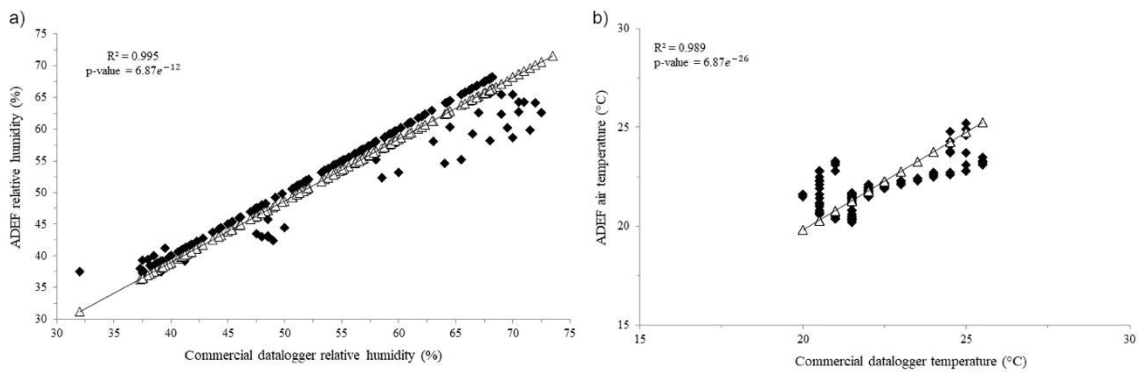

Figure 19-3 Distribution of (A) relative humidity (%) and (B) air temperature (°C) values obtained by both dataloggers (ADEF = white triangles and commercial = black rhombuses).

19.2.3 Analytical and uncertainty analysis for stage 1

Through the information provided by the manufacturers and the reference variable measured by the commercial datalogger (forced-ventilation oven: temperature - 52.30°C; room with the temperature controlled: air temperature - 22.02°C and relative humidity - 54.85%), an uncertainty budget (Type A and Type B) associated with ADEF measurements was determined. The results of the ADEFs standard uncertainty, calculated from the variables measured in stage 1 are shown in Table 19-1.

Table 19-1 Uncertainty budget of the sources needed to determine the standard uncertainty associated with air temperature (AT, °C) and relative humidity (RH, %) measured by the ambient sensor (DHT22), and temperature (°C) measured by thermal sensors (DS18B20) in the stage 1.

Parameter	Parameters	Value	Type	Distribution	Divisor	Standard uncertainty[b]
AT	Repeatability	-	A	Normal	1	0.0317
	Accuracy[a]	0.5	B	Rectangular	$\sqrt{3}$	0.0251
	Standard resolution[a]	0.1	B	Rectangular	$\sqrt{3}$	0.0757
RH	Repeatability	-	A	Normal	1	0.0781
	Accuracy[a]	2.0	B	Rectangular	$\sqrt{3}$	0.0295
	Standard resolution[a]	0.1	B	Rectangular	$\sqrt{3}$	0.0673
Thermal sensor 1	Repeatability	-	A	Normal	1	0.0068
	Accuracy[a]	0.5	B	Rectangular	$\sqrt{3}$	0.0295
	Standard resolution[a]	0.1	B	Rectangular	$\sqrt{3}$	0.0178
Thermal sensor 2	Repeatability	-	A	Normal	1	0.0058
	Accuracy[a]	0.5	B	Rectangular	$\sqrt{3}$	0.0289
	Standard resolution[a]	0.1	B	Rectangular	$\sqrt{3}$	0.0144

[a]manufacturer specifications, [b]determined from data measured by ADEF more manufacturer specifications and reference value measured by commercial datalogger.

During the stage 1, the values measured by the ADEF did not differ from the reference value, so there was no need for bias correction for ADEF measurements. There was a high quality of the statistical models between the data measured from the ADEF and the commercial datalogger. In addition, the ADEF showed low values of uncertainty associated with measurements (Table 19-2).

Table 19-2 Uncertainties budget of ADEF associated with air temperature (AT, °C) and relative humidity (RH, %) measured by the ambient sensor (DHT22), and temperature (°C) measured by thermal sensors (DS18B20) in the stage 1.

Parameter	Combined standard uncertainty	Relative standard uncertainty	Expanded standard Uncertainty
AT	0.1813	0.0162	0.3554
RH	0.1340	0.0049	0.2629
Thermal sensor 1	0.1361	0.0076	0.2386
Thermal sensor 2	0.1091	0.0040	0.2138

19.3 STAGE 2 – EVALUATION IN THE FIELD

In the stage 2, the ADEF measured 40,320 data of environmental variables (RH, AT, SST, and BGT). The first measurements of each component were excluded (in total 220 excluded data), so the total number of usable measurements after pre-processing was 40,100 data. The hourly variations of relative humidity and air temperature for the region (meteorological station) and experimental area (ADEF) are shown in Figure 19-4. Although there were variations between the measurements of environmental variables from the meteorological station and the ADEF, the measured variables showed the same pattern and a high correlation (relative humidity: $r = 0.93$; $p = 4.5e^{-13}$, and air temperature: $r = 0.91$; $p = 1.6e^{-11}$). Furthermore, the environmental variables measured by the ADEF showed low dispersion (Figure 19-5) associated with low relative standard uncertainty (Table 19-3).

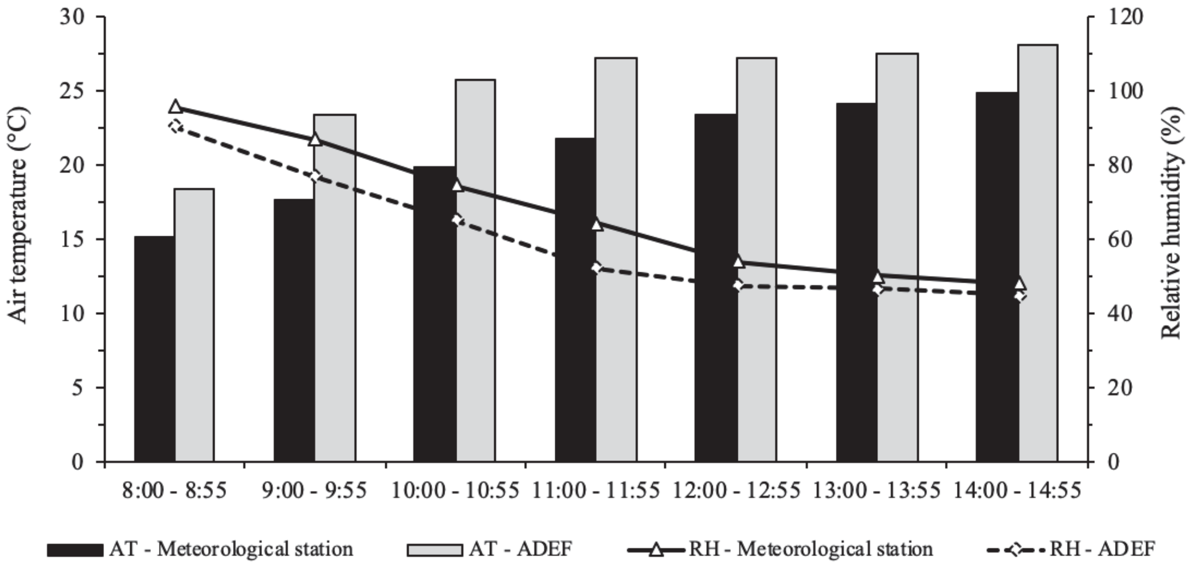

Figure 19-4 Hourly averaged data from environmental variables measured by the ADEF and meteorological station (Piraquara/PR - Simepar) during the evaluation in the field.

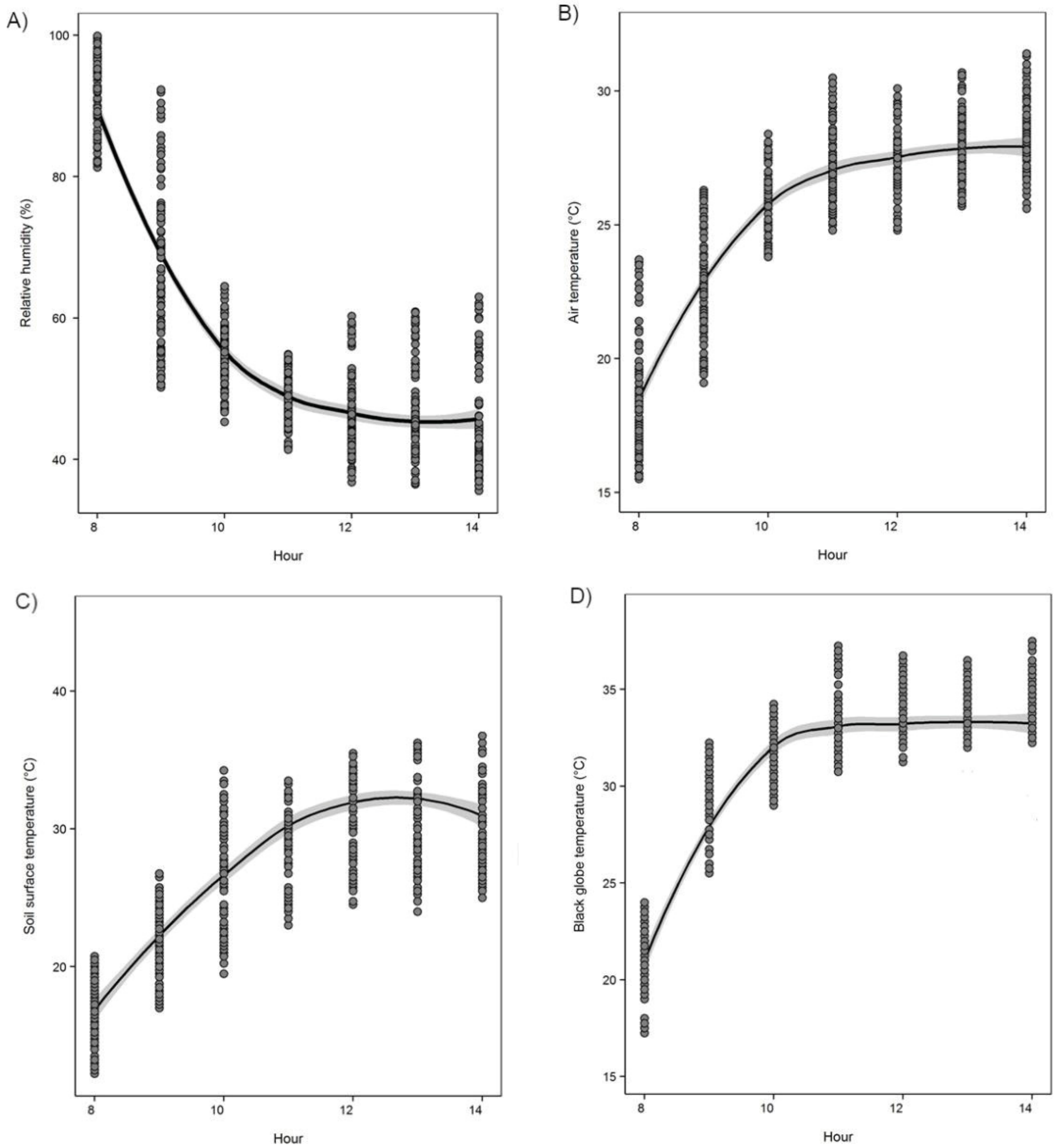

Figure 19-5 Hourly distribution of environmental variables measured by the ADEF (A) relative humidity (%), (B) air temperature (°C), (C) soil surface temperature (°C), and (D) black globe temperature.

Table 19-3 Mean values, confidence interval (CI = 95%), and relative standard uncertainty (RSU) associated with relative humidity (%), air temperature (°C), soil surface temperature (°C), and black globe temperature (°C) measurements during the evaluation in the field.

Relative humidity					Air temperature				
Hour	Average	CI		RSU	Hour	Average	CI		RSU
		lower	upper				lower	upper	
8 a.m.	90.56	88.99	92.13	0.0262	8 a.m.	18.47	17.88	18.65	0.0279
9 a.m.	77.07	65.52	68.62	0.0364	9 a.m.	23.35	22.97	23.74	0.0246
10 a.m.	65.23	53.73	56.72	0.0213	10 a.m.	25.77	25.39	26.16	0.0158
11 a.m.	52.32	46.84	49.80	0.0160	11 a.m.	27.20	26.82	27.58	0.0155
12 p.m.	47.66	46.15	49.17	0.0294	12 a.m.	27.26	26.87	27.65	0.0155
1 p.m.	46.68	45.16	48.20	0.0318	1 p.m.	27.59	27.20	27.97	0.0159
2 p.m.	44.99	43.48	46.50	0.0364	2 p.m.	28.07	27.69	28.46	0.0169
Soil surface temperature					Black globe temperature				
Hour	Average	CI		RSU	Hour	Average	CI		RSU
		lower	upper				lower	upper	
8 a.m.	17.05	16.26	18.00	0.0303	8 a.m.	21.67	19.63	20.84	0.0388
9 a.m.	21.74	20.85	22.63	0.0273	9 a.m.	29.28	28.65	29.77	0.0284
10 a.m.	26.21	25.32	27.10	0.0280	10 a.m.	32.05	31.49	32.61	0.0142
11 a.m.	30.60	29.72	31.49	0.0304	11 a.m.	33.23	32.68	33.79	0.0149
12 p.m.	31.87	30.97	32.76	0.0361	12 p.m.	32.88	32.32	33.44	0.0171
1 p.m.	31.76	30.88	32.65	0.0373	1 p.m.	32.86	32.30	33.41	0.0194
2 p.m.	31.15	30.26	32.04	0.0377	2 p.m.	33.50	32.94	34.06	0.0200

20 DISCUSSION

The estimated cost of each ADEF was $30 USD (excluding the cost of labor). The use of low-cost components, a microcontroller, and open-source software, allowed us to develop a datalogger with the same function and a cost of 60% lower than the commercial datalogger ($74.99), used as a reference system in this study. The ADEF had a low uncertainty and showed a high coefficient of determination with the commercial datalogger. That is why we believe that ADEF can easily be applied in researches to measure environmental variables at a farm level. The low cost provides an opportunity for researchers to purchase and implement devices in order to obtain a large database of several environmental variables. Therefore, measure environmental variables in different conditions can assist to develop predictive models that could also help to prevent or mitigate the effect of hot or cold stress (Wang et al. 2018). Thus, with local measures of environmental variables, a farmer can adopt and implement appropriate solutions to protect their animals (Herbut et al. 2018).

The ADEF was accurate in measuring environmental variables with a sufficient density of data to provide knowledge about the thermal environment in animal production systems. The thermal environment is one of the main factor to promote discomfort in farm animals, so it is essential to prevent the occurrence of potential stressor factors since, for efficient production, the animals need to remain within a thermal comfort zone (De Rensis et al. 2015; Sejian et al. 2018). The total amount of data collected by the ADEF (stages 1 and 2) generated a large and complex database in relatively little time. The spatial discretization achieved with the ADEF data in the field test will allow future data analytical techniques to explore how livestock respond to changes in climatic conditions. Analytical techniques such as data mining have been used to determine how environmental variables affect farm animals, for example, through an environmental database it is possible to classify heat waves (Vale et al. 2010), and to predict when there will be a higher chance of high mortality in laying hens (Riquena et al. 2019). Pattern extraction by data mining from large and complex databases allows building of the decision-making process. Low-cost devices (e.g., ADEF) can assist in decision making, as they offer an opportunity for more complete research to be carried out.

The ADEF recorded the highest average values of air temperature and lowest values of relative humidity when compared to data from the meteorological station. However, the overall evaluation indicated its performance followed the same pattern as the meteorological station, with a high correlation. The high correlation between the data is an important factor since the station as it is part of a state institutional network of meteorological measurements receives periodic monitoring and maintenance. In addition, the thermal environment of livestock production systems has a wide degree of variability (Schüller and Heuwieser 2016) and environmental variables should be measured at the farms (Shock et al. 2016). It is common in research and on commercial farms to estimate the thermal comfort of animals through environmental variables measures from the nearest meteorological station. However, studies have shown that the use of environmental variables measured at the meteorological stations nearest to the farms is not appropriate for estimating the thermal comfort of animals, as they can underestimate both the magnitude and duration of thermal stress (Ouellet et al., 2019; Schüller et al., 2013). So, we highlight that the ADEF is a good alternative for researches to better understand the microclimatic variability of an interest area.

Although the World Meteorological Organization (2018) states that low-cost sensors are not currently substitutes for reference instruments, in the current study we showed that the ADEF provided reliable data and presented low uncertainty associated with measurements. These values may be related to the accuracy of the sensor models. Studies in the field show that the accuracy of low-cost sensors depends on the model (Tagle et al. 2020). Data quality is an important trait to ensure the reliability of the results and to minimize the spread of uncertainty over an experimental period. In addition, it is important that environmental measurements are constant and consistent throughout the day, to ensure the receptivity of the results. Low-cost devices can assist with decisions, and through the incorporation of data science techniques (e.g. data mining) it will be possible to integrate better technologies in the future (World Meteorological Organization 2018).

The ADEF is a versatile device that can be easily used in environments ranging from grazing areas to barns (e.g. free-stall, compost barn, and broiler house); the consistency and low uncertainty of the measured data will contribute to better understanding the thermal environment at the farm level. Through the data measured by the ambient sensor of ADEF, it will be possible to determine the temperature and humidity index (THI); while, with a thermal sensor (DS18B20) fixed at the center of a hollow black sphere (Vega et al. 2020) it will be possible to measure the black globe temperature (BGT). Thus, associating the BGT with the ambient sensor data, allow the determination of the black globe humidity index (BGHI). THI is one of the most commonly used animal comfort indexes in the world (Habeeb et al. 2018), but it is indicated for indoor environments, while the BGHI is an efficient index of thermal stress in open areas (Magalhães et al. 2020). In addition, with the other thermal sensor, it is possible to measure the soil surface temperature (as in this study), as well as the temperature of the bed in barns and broiler houses. This measure can be used to infer the life quality of farm animals, for example, cows identify more comfortable regions to perform rest behaviors (Peixoto et al. 2019); since areas used for lying behavior are influenced by the soil surface temperature (de Sousa et al. 2021b). Furthermore, in broiler houses, bed temperature is a factor that directly influences productivity, because it contributes to ammonia volatilization (Vale et al. 2016) and can promote a high incidence of dermatitis (Bessei 2006).

The accumulation of dust and dirt as a negative effect on the response time of the sensors was reported in other studies (Caquilpán et al. 2019; Ramirez et al. 2018).

Dust in barns and broiler houses are still a major barrier to the implementation of advanced measurement systems. An active purge system (puffs of air) to remove/prevent dust accumulation or “self- cleaning” surface coatings may have potential to reduce dust related problems for livestock facility sensors (Ramirez et al. 2018). In our study, dust accumulation was not a problem, as the sensors were placed under a meteorological shelter. This shelter protects the sensors from direct exposure of solar radiation, prevents the accumulated dust from reaching the sensors, and, due to the fact that it can be disassembled, facilitates hygiene, being a low-cost alternative to circumvent the dust problem.

Integration of monitoring technologies in the last decades (Tullo et al. 2019) has been fundamental in the development of new ways to understand factors that directly affect air quality (Caquilpán et al. 2019; Tagle et al. 2020), soil humidity (Senpinar 2019), physiological measures (Eigenberg et al. 2008), and behavioural (Ngo et al. 2020) in farm animals. As we showed in this study, the ADEF could represent an economical solution to obtain accurate and relevant data regarding the thermal environment on farms, and thus to infer about the quality of life of the animals. With the help of the ADEF, we can measure four variables that can impair the thermal comfort of farm animals. Our results, as well as those from new research involving ADEFs, can help the development of warming systems, helping policymakers to understand the likely economic impact of climate change on livestock.

21 CONCLUSION

The ADEF presented reliable results and was efficient in measuring environmental variables with low uncertainty. The uncertainty analysis presented in this study provides a framework for executing the uncertainty associated with similar measurement systems and determining whether a significant bias of measurements existed. The ADEF proved to be extremely versatile and can be easily applied as a low-cost tool to measure environmental variables. In addition, ADEF development is a necessary advance in precision livestock farming, once it had the capacity to measured and store a large and complex database in relatively little time.

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Declarations:

Conflict of interest: The authors declare that they have no competing interests.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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23 CHAPTER IV - CLASSIFICATION OF ENVIRONMENTAL FACTORS POTENTIALLY MOTIVATING FOR DAIRY COWS TO ACCESS SHADE

Matheus Deniz[1*], Karolini Tenffen de Sousa[1], Isabelle Cordova Gomes[1], Marcos Martinez do Vale[1], João Ricardo Dittrich[1]

[1]Programa de Pós-Graduação em Zootecnia, Departamento de Zootecnia, Universidade Federal do Paraná, Curitiba, Brazil.

[2]Laboratório de Inovações Tecnológicas em Zootecnia (LITEZ – UFPR), Departamento de Zootecnia, Universidade Federal do Paraná, Curitiba – PR, Brazil.

Short title: Motivating environmental factors for dairy cows to access shade

*Corresponding author:
Matheus Deniz; ORCID: 0000-0001-8079-0070
E-mail: matheus-utfpr@hotmail.com;

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Abstract

The aim of this Research Communication was to apply the data mining technique to classify which environmental factors have the potential to motivate dairy cows to access natural shade. We defined two different areas at the silvopastoral system: shaded and sunny. Environmental factors and the frequency that dairy cows used each area were measured during four days, for 8h each day. The shaded areas were the most used by dairy cows and presented the lowest mean values of all environmental factors. Solar radiation was the environmental factors with most potential to classify the dairy cow's decision to access shaded areas. Data mining is a machine learning technique with great potential to characterize the influence of the thermal environment in the cows' decision at the pasture.

Key words: animal distribution; behavioural pattern; decision tree; pasture; precision livestock farming

24 INTRODUCTION

The general public prefers production systems that promote heat abatement for farm animals, like shade on pasture and fans in indoor housing (Cardoso *et al.,* 2018). However, heat stress is one of the main challenges of grazing cows, as animals on pasture are constantly submitted to great environmental variability. Nowadays, new approaches to data analysis (fuzzy logic, artificial neural networking and data mining) can help researchers interpret large databases, improving livestock farming through a better understanding of the production system. Pattern extraction by data mining potentially allows accurate decision-making. Data mining tasks have been used in several dairy production research areas in an attempt to detect problems such as dystocia and calving difficulty (Zaborski *et al*., 2017, 2018), mastitis (Sharifi *et al*., 2018) and factors affecting cow reactivity during milking (Neja *et al*., 2017). However, most such research has focused on confined animals and only a few papers have applied this technique to analysis of animal behaviour raised on pasture. Based on this, we hypothesized that data mining can be applied for rule extraction and to identify potentially motivating environmental factors for grazing dairy cows to access shade.

25 MATERIAL AND METHODS

The experiment was conducted in accordance with guidelines laid down by the Animal Ethics Committee of the Universidade Federal do Paraná and national legislation.

25.1 EXPERIMENTAL AREA AND MANAGEMENT

The study was carried out on a commercial dairy farm in southern Brazil. Data collection was performed during summer (southern hemisphere). The experimental area had 4 paddocks (1.500m²/ paddock), and each one was composed of a silvopastoral system (SPS). The silvopastoral system provided a total shaded area of 5m²/ animal in each paddock and a sunny area of 33m²/ animal. The cows were moved daily to a new paddock. The paddocks and SPS distribution were uniform, allowing us to evaluate one paddock per day.

25.2 ANIMALS AND FREQUENCY AT THE SHADED AND SUNNY AREAS

Lactating Jersey cows (n=39), with similar coat colour (light brown), and weight (mean ± SD) of 450 ± 50kg were observed during four days, for approximately 8h each day (from 9:00 to 16:50). As the proposal of this study was to classify the cows' decision in relation to environmental factors regardless of behaviour, we evaluated the frequency of dairy cows located in different areas of the SPS. The frequency of animals in shaded and sunny areas was recorded by scan sampling at 10 min. intervals. The cow was considered to be in the shaded area when more than 50% of her body was in the shade of the tree. The cow was considered to be in a sunny area when more than 50% of her body was in the sun.

25.3 ENVIRONMENT EVALUATION

Environmental factors of air temperature (AT, °C), relative humidity (RH, %), solar radiation (SR, W/m²), and wind speed (WS, m/s) were measured in each area (shaded and sunny) of the SPS (detailed in the online Supplementary File). Data collection was carried out from 9:00 to 16:50 with intervals of 10 min., at a height of 1.3m from the ground, which corresponds to the height of the center of mass of Jersey adult cattle.

25.4 DATA MINING AND STATISTICAL ANALYSIS

Animal frequency at the areas and environmental data were used to build a database with 29320 observations and 10 variables, one being the classification (online Supplementary Table S1). The database was built with each observation (frequency at the areas and environmental) synchronized by date and time of day. Data mining was performed with aid of the software Waikato Environment for Knowledge Analysis (WEKA®, 3-4), which classifies the data and builds a classification tree using the J48 algorithm. Model accuracy, as well as class precision, were calculated by a confusion matrix (online Supplementary Table S2). In order to confirm the level of agreement of the data sets and classification accuracy, the Kappa statistical method was used (online Supplementaty Equation 1).

As confirmatory analysis, the data (frequency at the areas and environment) were analyzed by generalized linear models and submitted to the Spearman correlation test by statistical software R. In all models, areas were used as fixed effect, while animals, days and hours were fixed as random effects. The confidence intervals were estimated using Type II Wald chi-square tests and the fit of the model was given by a likelihood-test. The fitness of the models was tested by inspecting the residual in the graphs, a line of best fit. The normality of the random facts was given by quartile plot means with a confidence interval of 95%.

26 RESULTS

The data mining model correctly classified 87% of the instances with substantial accuracy (Kappa=0.73; Landis and Koch, 1977) and provided precision in classifying the location classes, shaded (0.96) and sunny (0.74). The model showed five classification rules, these being two rules for cow's decision to access sunny areas, and three rules for accessing shaded areas. The solar radiation (root node of the classification tree) was the most important environmental factor to classify the cow’s decision (Figure 26-1).

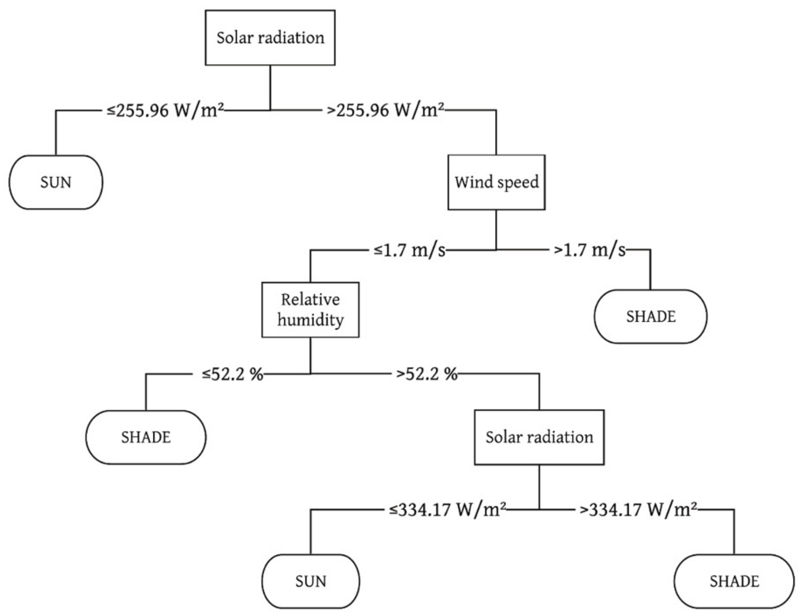

Figure 26-1 Classification tree for dairy cows’ decision-making in the silvopastoral system.

The environment of conditions (shaded or sunny) influenced (Z=15.449, p<0.001) the areas used by animals, the highest frequency (70%) being that recorded in the shaded areas. There was a difference in air temperature (F=3.419, p<0.001, Figure 26-2a) and solar radiation (F=25.716, p<0.01, Figure 26-2c) between the shaded and sunny areas. In addition, air temperature and solar radiation were correlated (r=0.63; p<0.05). Shaded areas exhibited a numerically higher relative humidity but this differecne was not significant (F=1.864, p>0.05, Figure 26-2b). Wind speed did not differ between the area (F=1.213, p>0.05, Figure 26- 2d).

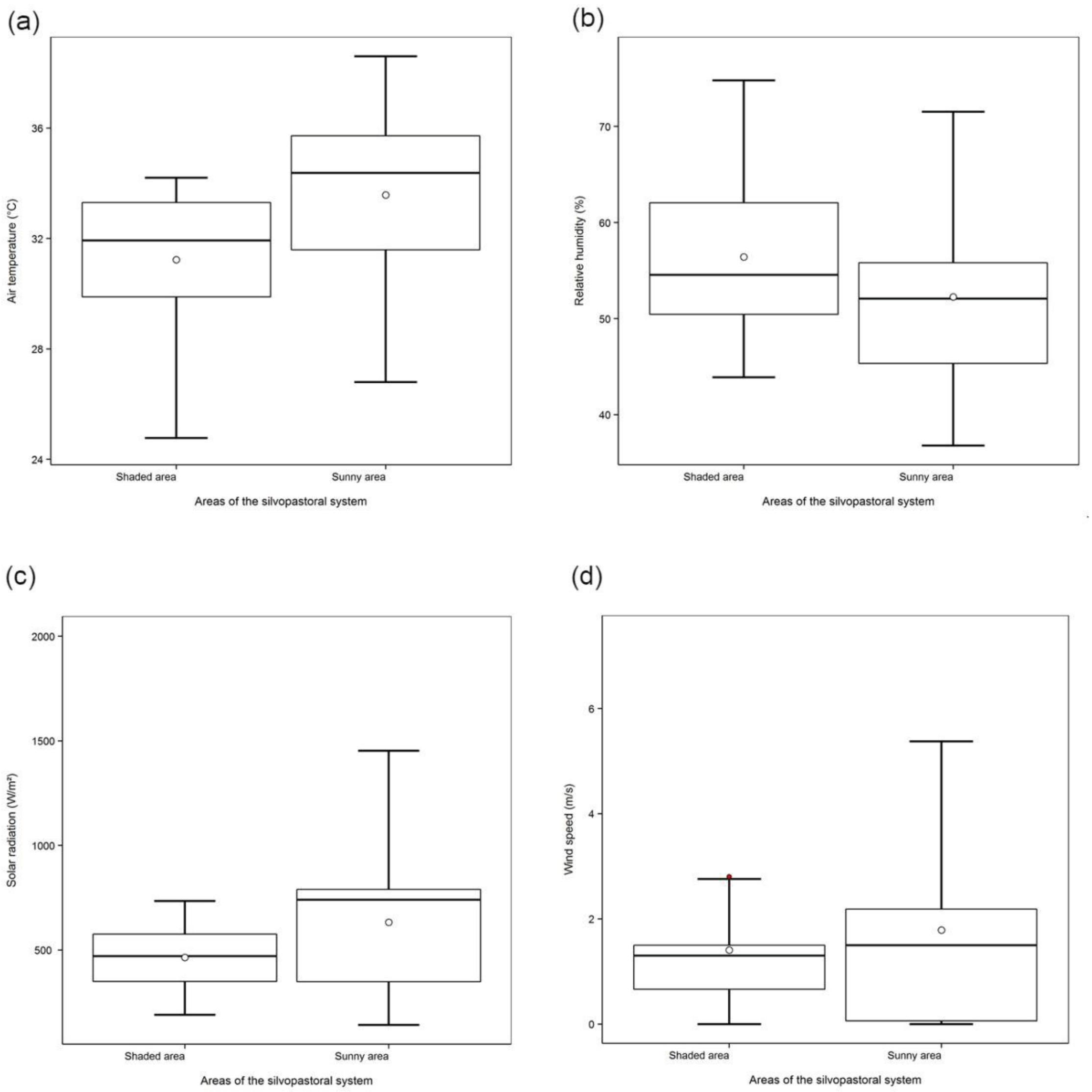

Figure 26-2 Variation of the environmental factors air temperature (a), relative humidity (b), solar radiation (c), and wind speed (d) by areas (shaded and sunny) of the silvopastoral system.

The critical period for solar radiation (727W/m^2) was in the afternoon between 12:00 and 12:50. Both solar radiation and shade use increased during the morning and until 12:50. The shade use of dairy cows increased by an average of 8.5% per hour during this time (Figure 26-3).

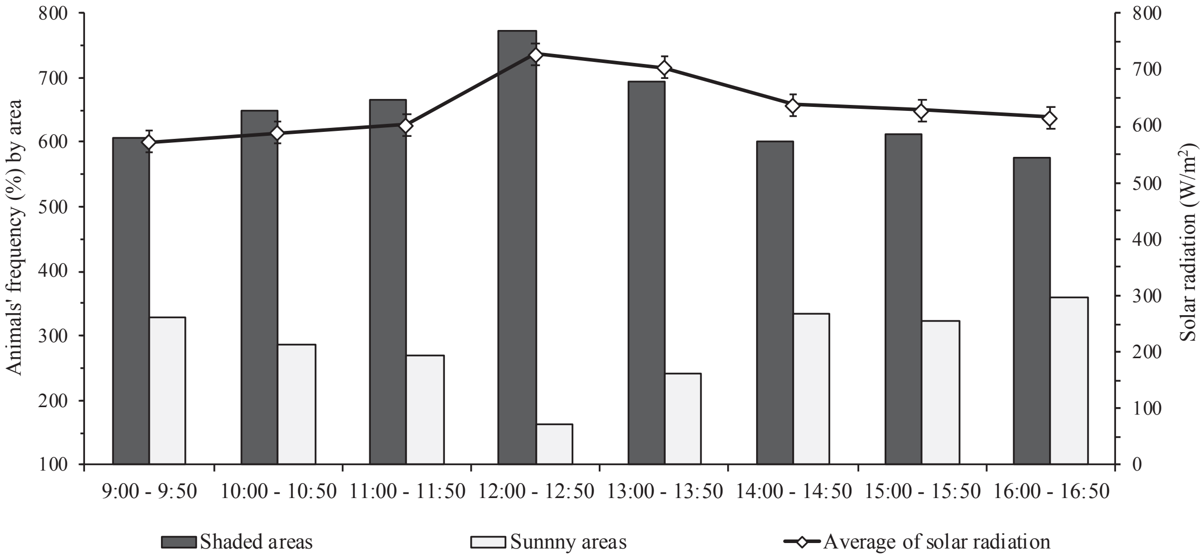

Figure 26-3 Animals' frequency by areas (shaded and sunny) in relation to solar radiation.

27 DISCUSSION

This is the first study that has applied data mining in a classification task to identify the interaction between dairy cow decision and environmental factors in pasture systems. In our study, solar radiation was the most reliable environmental factor to indicate cows' decision to access shaded or sunny areas. This does not mean that the classification tree excluded the influence of air temperature in cows' decisions, but in the mathematical space, solar radiation was more important. The correlation between solar radiation and air temperature may justify why the algorithm did not consider the air temperature for building the classification tree. As an attribute of simplification, the model uses the minimum information necessary for classifying the attributes (Buczak and Guven, 2016), since variables that present a significant correlation can compromise the model development. Not considering redundant attributes is a task that improves the model accuracy.

Both variables, solar radiation and air temperature, were lower in the shaded areas, and they are important when assessing the animals' thermal comfort. The individual effect of environmental factors on dairy cow behaviour is well known, but studies about the interaction of environmental factors are limited (Sejian *et al.*, 2018;

Tullo *et al.*, 2019). In livestock, thermal comfort indices help to interpret the complex interactions between the physical (environment) and biological (animal) components, resulting in a variety of responses, which must be interpreted according to the animal species and individual characteristics. As an example, the temperature and humidity index is the main thermal comfort index used in animal production (eg at pasture see Sharpe *et al*., 2020 and in confinement see Mcdonald *et al*., 2020). However, a disadvantage is that it only considers these two variables (Sejian *et al*., 2018). When assessing the thermal comfort of animals raised on pasture, solar radiation must be considered, as it is one of the main environmental factors that trigger thermal stress (Magalhães *et al*., 2020).

To respect ecological limits and provide better welfare conditions for farm animals, we need to provide elements to mitigate adverse situations. Whilst in confinement management the elements are specific and controllable, like fans and sprinklers, in pastoral environments natural shade is the most important element to mitigate the effects of environmental factors. In our study, the cows remained longer in the shaded (70%) than sunny areas. Shaded areas present better microclimatic conditions and cows remain longer in these areas during the summer (Deniz *et al*., 2020), even on days with relatively low levels of solar radiation (Schütz *et al*., 2010). In the winter, cows could also use shade when motivated to do so, either to seek protection from the sun in the middle of the day or to rest (de Sousa *et al*., 2021). We only evaluated cloudless days, but we could observe that as solar radiation increased during the morning, the animals moved to shaded areas. Besides shade providing a better microclimate for the animals, raising animals in shaded pasture meets consumer expectations for animal production systems closer to natural (Cardoso *et al.,* 2018).

Pattern recognition is an important tool to improve livestock management, as this is based on the extraction of characteristics that help to classify a study object (Theodoridis and Koutroumbas, 2003). From the categorization of data (in this case animal behaviour) the most probable hypothesis (here, shade use) can be found within a set of hypotheses. Thus, models developed by data mining help in the interpretation of complex databases, and assist the farmers in decision making to improve the animal's thermal comfort and reduce economic losses. However, obtaining a large database of pasture areas remains a challenge, as generally the number of animals per area is lower when compared to confinement. In addition, pastoral environments are complex systems with great environmental variability, since many obstacles hinder the total control of these open systems. Thus, we strongly suggest that future research uses data mining in environmental science and in the behaviour of animals raised on pasture to expand our knowledge on more sustainable systems models.

In conclusion, the data mining (machine learning technique) allowed the development of a mathematical model to extract patterns of environmental factors that influence the decision of dairy cows to access determined areas. Solar radiation was the environmental factor with the greatest potential to classify the decision of dairy cows to access shaded areas. Through the pattern found in our study, we suggest that studies evaluating thermal comfort of dairy cows on pasture areas should use indices that consider solar radiation.

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29 SUPPLEMENTARY FILE CHAPTER IV

MATERIALS AND METHODS

EXPERIMENTAL AREA AND CLIMATE PATTERN

This work was carried out in a silvopastoral system (SPS) on a commercial dairy farm in southern Brazil. Data collection was performed during summer (southern hemisphere); in four consecutive days with high temperatures, high solar radiation, and low cloudiness. According to Köppen classification, the climate of the region is subtropical humid mesothermic (Cfa) and presents hot summers with average annual temperatures between 18 and 20°C and relative humidity between 63 and 84% (INMET *et al*., 2009; Alvares *et al*., 2013).

The experimental area had 4 paddocks (1.550m²/ paddock) where each one was composed of a silvopastoral system. This system consisted of native trees (approximately 8 meters high) planted in wood with a distance of 14 meters, and provided a total shaded area of 5m²/ animal in each paddock (determined by Shading Vegetation Index) and a sunny area of 33m²/ animal in each paddock. At the farm, animals are raised permanently on pasture, mainly composed of plant species of *Axonopus catarinenses, Arachispintoi spp.* and *Paspalum notatum*. The pasture is managed under Voisin's Rotational Grazing system whereby animals are moved daily to a new paddock. Thus, as the paddocks and SPS distribution were uniforms, this allowed us to evaluate one paddock per day.

ANIMALS AND FREQUENCY AT THE SHADED AND SUNNY AREAS

Lactating Jersey cows (n = 39), with similar coat colour (light brown), weight (mean ± SD) of 450 ± 50kg were observed during four days, for 8h each day (from 09:00 to 16:50). All observations were performed in an area already known by the animals and began after the last animal entered at the paddock. To minimized research bias, after milking morning, animals were handled by farmers to the experimental area. Frequency of animals in each area (shaded and sunny) was recorded by scan sampling of 10 min. intervals (Altmann, 1974). The cow was considered to be in the shaded area when more than 50% of her body was in the shade of the tree. The cow was considered to be in the sunny area when more than 50% of her body was in the sun (Kendall *et al*., 2006; *Giro et al*., 2019). All observations were made by researchers previously trained and with knowledge in the area of animal behaviour; in order to not interfere with the animals' behaviour, the observations were performed outside of the paddock with a safe distance. The reliability of simultaneous observations of a given individual by the observers reached 94.2% before the beginning of the data collection.

ENVIRONMENT EVALUATION

During the experimental period, environmental factors were collected in 120 points [fifteen in each area (shaded and sunny)]. Thus, in order to avoid temporal variations between the areas, data collection was carried out simultaneously in both areas. In shaded and sunny areas of the SPS, the following environmental factors were measured: air temperature (AT, °C), relative humidity (RH, %), solar radiation (SR, W/m²) and wind speed (WS, m/s).

Air temperature (°C) and relative humidity (%) measurements were performed (with solar radiation shield) with a thermo-hygrometer (humidity 0-100% scale; ± 2.5% accuracy; 0.1% resolution; temperature, -30 to 100°C scale; ± 0.8°C accuracy; and 0.1°C resolution). The solar radiation measurement was performed with a pyranometer (0 to 4000W/m²; ± 4% accuracy). Wind speed was measured with a thermo-anemometer (0.4 to 20 m/s scale; ± 2% accuracy). Data collection was carried out from 9:00 to 16:50 at a height of 1.3m from the ground (height average of the center of mass of Jersey adult cattle) with intervals of 10 min., and averages were generated every 1 h.

DATA MINING AND STATISTICAL ANALYSIS

Animal frequency at the areas and environmental data were used to build a database with 29320 observations and 10 variables, one being the classification (Supplementary Table 29-1). The database was built with each observation (frequency at the areas and environmental) synchronized by date and time of day. Data mining technique was applied following CRISP-DM methodology (Klein *et al*., 2020).

Supplementary Table 29-1 Summary of data and variables of the final database.

Nº	Variable	Unit	Nº	Variable	Unit
1	Day[A]	Numeric	6	Air temperature	°C
2	Hour[B]	Numeric	7	Relative humidity	%
3	Categorized time[C]	Numeric	8	Solar radiation	W/m²
4	Scan[D]	Numeric	9	Wind speed	m/s
5	Animals ID[E]	Numeric	10	Areas: shaded/ sunny[F]	Class

[A]collection days; [B]hours of data collection (range: 1 to 8); [C]categorization of observation hours in period (morning and afternoon); [D]observations of frequency at the areas in each 10min.; [E]individual identification by animal; [F]nominal classification of each event based on the area used by animal.

Data mining was performed with the software Waikato Environment for Knowledge Analysis (WEKA®, 3-4), which classifies the data and build a classification tree using the J48 algorithm, an implementation of the algorithm C4.5 that is a supervised machine learning tool. The J48 algorithm generates a model with semantic rules using the minimum information required for classification. The model result is expressed graphically in the form of an inverted tree; the first attribute is the one with the highest classification power (root node). From the root node, semantic rules are expressed as body ➔ head. The rules body are logic connectors (≤, >, and =) called as nodes that express the connection between the features that are capable to classify an event. The classification from a rule is the head that is represented in the graphic tree as the leafs. Each branch in the classification tree is one rule with their connectors in the body and a class on the head.

Classification tree was generated by ranking the cow's frequency at the areas (shaded or sunny), according to the environmental factors. The best model selection was based on the model accuracy, the precision of classes, and the interpretation of classification rules by experts with the minimum requirement of three years of expertise. In the analysis, were applied a ten-fold cross-validation, available in the J48 algorithm. Model accuracy, as well as class precision, were calculated by a confusion matrix (Supplementary Table 2). The class precision ranges from zero to one and expresses the relation of true positive and true negative classifications in a specific class. The model accuracy expresses the percentage of instances that were correctly classified.

Supplementary Table 29-2 Confusion matrix representation

Class	Predict as C_+	Predict as C_-	Class precision	Model accuracy[A]
C_+	True positives (T_p)	False negatives (F_n)	T_p/ (T_p + F_n)	[T_p + T_n)/ N] x 100
C_-	False positives (F_p)	True negatives (T_n)	T_n/ (F_p + T_n)	

[A]N is equal to the number of instances in the test set.

In order to confirm the level of agreement of the data sets and classification accuracy, the Kappa statistical method was used (see more information in: Sim and Wright 2005; McHugh 2012) was determined by equation (1) developed by Cohen (1960). In this study, when describing the relative strength of agreement associated with kappa statistics, the labels proposed by Landis and Koch (1977) were used. The relative strength values indicate: ≤0: poor; 0.00 – 0.20: slight; 0.21 – 0.40: fair; 0.41 – 0.60: moderate; 0.61 – 0.80: substantial; and 0.81 – 1.00: almost perfect.

$$\mathrm{K} = \frac{\mathrm{P_0} - \mathrm{P_c}}{1 - \mathrm{P_c}} \quad (1)$$

Where:
K is the kappa statistical,
P_o is the proportion of observed agreements and,
P_c is the proportion of agreements expected by chance.

As confirmatory analysis, the data (frequency at the areas and environment) were submitted to the normality test (Shapiro-Wilk), analyzed by Generalized Linear Models (GLM) and submitted to the Spearman correlation test. Experimental design of environmental factors was composed of four replicates (paddocks), 120 experimental units (30 collection points by paddock), two independent variables (shade and sun) and four dependent variables (air temperature, relative humidity, solar radiation and wind speed) following the model:

$$Y_{ij} = \alpha_j + \beta_{ij} + e_{ij}$$

Were:

Y_{ij} are the microclimatic variables,

α_j are the fixed effect of the areas provided by the silvopastoral system,

β_{ij} is the random effect, *i* corresponds to days; *j* corresponds to hours, and

e_{ij} is the residual effect.

All analyzes were performed separately and each environmental factor obtained a GLM model. Gamma distribution and logarithmic bonding function were used for the environmental factors, at a 95% confidence level.

The analysis of frequency at the areas was composed of four repetitions (paddocks), 39 experimental units (animals), two independent variables (shade and sun) and the dependent variable was the frequency of events recorded in shaded and sunny areas. Poisson distribution at a confidence interval of 99% was used. Animals, days and hours were defined as random effects following the model:

$$Y_{ij} = \alpha_j + A_i + \beta_{ij} + e_{ij}$$

Were:

$Y_{ij}$is the cow's frequency at the areas,

α_j are the fixed effect of the areas provided by the silvopastoral system,

A_i is the random effect of animals,

β_{ij} is the random effect, *i* corresponds to days; *j* corresponds to hours, and

e_{ij} is the residual effect.

All analyzes were performed through the statistical software R (R Core Team 2019) and all statistical models were adjusted using the maximum likelihood-Laplace approximation method in the statistical package lme4 (Bates *et al*., 2015).

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30 CHAPTER V - SOCIAL HIERARCHY INFLUENCES DAIRY COWS' USE OF SHADE IN A SILVOPASTORAL SYSTEM UNDER INTENSIVE ROTATIONAL GRAZING

Matheus Deniz[a], Karolini Tenffen de Sousa[a], Matheus Fernando Moro[a], Marcos Martinez do Vale[a-b], João Ricardo Dittrich[a-b], Luiz Carlos Pinheiro Machado Filho[c], Maria José Hötzel[c]

[a]Laboratório de Inovações Tecnológicas em Zootecnia, Departamento de Zootecnia, Universidade Federal do Paraná, Curitiba 800035-050, Brazil.

[b]Departamento de Zootecnia, Universidade Federal do Paraná, Curitiba 800035-050, Brazil.

[c]Laboratório de Etologia Aplicada e Bem-estar Animal, Departamento de Zootecnia e Desenvolvimento Rural, Universidade Federal de Santa Catarina, Florianópolis 88034-001, Brazil.

*Corresponding author:
Matheus Deniz; ORCID: 0000-0001-8079-0070
E-mail: matheus-utfpr@hotmail.com;

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Abstract

The aim of this study was to evaluate the relationship between thermal comfort indicators and social hierarchy, and cows' location (shade or sun) and their diurnal behaviors in a silvopastoral system of a subtropical climate, covering the four seasons. We measured microclimatic variables (air temperature, relative humidity, wind speed, soil surface temperature) and cows' behaviors in two areas (shaded and sunny), as well as the influence of social hierarchy (dominant, intermediate, and subordinate) on cows' location (shade or sun). In addition, we determined the black globe-humidity index (BGHI) and radiant heat load (RHL) for both areas. Air temperature, wind speed, soil surface variables were lower in shaded areas, and relative humidity lower in the sunny areas ($p<0.05$). The shaded areas provided on average a 23% RHL reduction in cold seasons (autumn and winter), and 26% in hot seasons (spring and summer). For cows of all social categories the odds of drinking water decreased ($p<0.001$) for each additional BGHI unit increased. Dominant cows were less likely (~50%; $p<0.001$) of drinking water than intermediate and subordinate cows. In general, the odds of a cow lying in the sunny areas were 62% lower than in the shaded areas ($p<0.001$). However, in winter cows were less likely (75%) to perform comfort behaviors (idling and rumination lying down) in shaded areas than in the sunny areas ($p<0.01$). Furthermore, lying increased by 9% for each additional soil surface temperature unit. Dominant cows were more likely (~40%; $p<0.001$) to lie down in the shaded areas than intermediate and subordinate. In conclusion, the cows' location in the silvopastoral system was influenced by the black globe-humidity index and social hierarchy; in a situation of higher thermal challenge cows were more motivated to use shade, but dominant cows were more likely to use the shaded areas than other cows.

Key words: Animal welfare; Applied ethology; Grazing dairy herd; Heat abatement; Social behavior; Thermal comfort

31 INTRODUCTION

Conciliating trade-offs among livestock efficiency, maintenance of biodiversity, and ecosystem services will be the challenges for the coming decades. Good health and animal welfare are considered essential to maintain low environmental impacts in the livestock sector (Broom, 2017). Raising animals in well managed pasture-based systems can contribute to the mitigation of climate change through soil organic carbon sequestration (Seó et al., 2017; Stanley et al., 2018), and improve their welfare as these systems allow animals to express their natural behaviors (Charlton and Rutter, 2017; Crump et al., 2021). However, heat abatement for grazing cows is essential, given that under adverse conditions cows can present thermal stress (Kadzere et al., 2002). Besides economic impacts (St-Pierre et al., 2003), adopting mechanisms that mitigate environmental stressors in animal production may also address ethical concerns of consumers (Cardoso et al., 2018).

The benefits that silvopastoral systems (SPS) can bring to the environment (Barton et al., 2016; Castro and Fernández-Núñes, 2016; England et al., 2020), and animals' thermal comfort (de Sousa et al., 2021b; Deniz et al., 2020; Giro et al., 2019) are well reported in the literature. Yet, raising cattle in silvopastoral systems is a challenge, due to the complexity of managing simultaneously the pasture, the trees and the animals in the same area (Chará et al., 2019; Mee and Boyle, 2020). The interest to understand and popularize the silvopastoral systems has motivated research around the world. However, some studies that evaluated the thermal comfort in silvopastoral systems did not consider the animals (Deniz et al., 2019; Karvatte et al., 2016; Pezzopane et al., 2019). Also, some studies focused on the animals but did not consider herd effects (Améndola et al., 2019; de Sousa et al., 2021b; Giro et al., 2019). The gregarious characteristic of cattle influences the behavior of individuals in the group, and should not be excluded from the studies. The way bovines use the resources available in the environment depends on environmental factors such as the location of the shade (Stivanin et al., 2019), of the water (Coimbra et al., 2012), and of the food (Bica et al., 2020, 2019) within the paddocks, as well as social rank of the individuals (Bica et al., 2020, 2019; Coimbra et al., 2012). Social hierarchy influences cattle strategies to cope with thermal stress, given that dominant cows have priority in accessing resources such as water and space to rest (Coimbra et al., 2012; di Virgilio and Morales, 2016; Takanishi et al., 2015). Thus, animals adopt different strategies to deal with environmental challenges; for example, in feedlots low social ranking cows shift their drinking behavior throughout the day, to avoid hottest hours and competition (McDonald et al., 2020). Also, in pasture-based system, dominant cows are able to monopolize the water trough when it is located in a narrow space (Coimbra et al., 2012), and subordinate heifers perform grazing activities more frequently when dominant heifers are ingesting supplement (Bica et al., 2020). One study evaluating the social behavior of cows raised in a silvopastoral system found that the group of animals that remained in the shaded areas showed social stability and expressed more socio-positive behaviors, compared to cows grazing on a monoculture without trees (Améndola et al., 2016).

The knowledge of behavioral strategies of dairy cows within a silvopastoral systems can help us to better understand the cows' thermodynamics in these systems. The silvopastoral systems offer the animals the possibility to choose between areas of shade or sun, so that cows are able to remain in the shade in the hottest hours of the day (Deniz et al., 2020), and in the sun when motivated to do so (de Sousa et al., 2021b). However, the social hierarchy of the herd may influence the behaviour of individuals in the herd. Thus, the aims of this study were: (1) to evaluate the influence of the silvopastoral system on the microclimate and thermal comfort indicators of shaded and sunny areas; (2) to compare the use of shade by cows of different social categories throughout the year; and (3) to evaluate the influence of cows' location, microclimate, social hierarchy, and season on diurnal behaviors of dairy cows.

32 MATERIALS AND METHODS

This study was approved by the Ethics Committee on Animal Use of "Universidade Federal do Paraná" under protocol number 083/2018, and it was performed in accordance with the ethics of animal experimentation.

32.1 LOCATION AND CLIMATE PATTERN

The experiment was carried out between March 2020 and February 2021 (covering the four seasons of the southern hemisphere) at the "Estação de Pesquisa Agroecológica – CPRA", of the "Instituto de Desenvolvimento Rural do Paraná", Paraná state, in Southern Brazil (25°26'41"S, 49°11'33"W). The climate of the region is characterized as temperate oceanic (Cfb) according to the Köppen's classification (Alvares et al., 2013; INMET, 2009). The region presents minimum temperature below

18°C and maximum above 22°C, with average monthly precipitation above 100mm (details are shown in supplementary Table S1).

32.2 EXPERIMENTAL AREA

At the farm, the pasture area was managed under Voisin's Rational Grazing (Pinheiro Machado, 2010; Voisin, 1974). In this system, the pasture area is divided into paddocks, and the herd enters to a new paddock twice a day (after each milking). In all paddocks water was available in round water troughs made of Polythene (120 cm diameter and 60 cm high and 500 L capacity; Tigre®, Joinville, SC, Brazil). Voisin's system is ecologically useful, sustainable and, a key solution for the problems faced by climate change (Schröter et al., 2015), as it improves the structure and productivity of the soil due to biocenosis; reduces the greenhouse gas emissions from the animals; decrease the fertilizer and pesticide use; and improves the health of the animals (Pinheiro Machado, 2010; Schröter et al., 2015; Seó et al., 2017). The pasture area selected for this study was composed by two experimental paddocks (2400m2) of a silvopastoral system with trees along the border fences. The silvopastoral system (SPS) was composed of pastures with natural shading due to the presence of trees in a single row along the border fences. The system was implemented 10 years before this study and had approximately twenty trees per paddock (Eucalyptus urograndis, Patagonula americana, Psidium cattleyanum, Eugenia uniflora, Campomanesia xanthocarpa, and Cordia trichotoma) in a single row, in a northeast-south-west orientation, with 30m between rows and 4m between trees. Trees height during the experimental period was on average 8m and the interception of solar radiation by the tree canopies was 86% in autumn, 78% winter, 87% spring, and 83% summer.

In the silvopastoral systems (100 trees/ha) we determined two different areas, shaded and sunny (Deniz et al., 2020), with approximately the same dimension by paddocks. The useful shaded area (9.8% of the paddock area = 10.7m2 of shaded per animal) was determined by the Shading Vegetation Index (SVI, equation 1). The shaded area available was enough for all animals (Schütz et al., 2014; Stivanin et al., 2019). The sunny area in the silvopastoral systems represents the percentage of the paddock area excluding the shaded area (90.2% of the paddock area = 98.4m2 of sunny per animal).

$$SVI = \left[\frac{\text{shaded area } (m^2)}{\text{total area of paddock } (m^2)}\right] \times 100 \qquad (1)$$

All paddocks were based on pasture mainly composed of plant species of Axonopus spp., Paspalum spp., Pennisetum spp., Cynodon spp., Trifolium spp., and Lolium spp. In the cold seasons (autumn and winter) the SPS had a pasture with an average height of 25cm; the pasture production was 763kg/DM/ha, and during the hot seasons (spring and summer) the pasture had an average height of 30cm; the pasture production was 1,276kg/DM/ha (mean percentage values of pasture quality by areas are shown in Supplementary Table S2). The amount of pasture available was enough for all animals (Aikman et al., 2008; Coffey et al., 2017).

32.3 EXPERIMENTAL PERIODS

The observation was carried out during four days per season, divided in two experimental periods (Figure 32-1). Period 1 was composed of measurements during two consecutive days on the optimum recovery of pasture (ORT; Machado Filho et al., 2011), and period 2 was composed by two consecutive days after the pasture rest. The ORT is the time needed for a new regrowth of the pasture, and in this study was approximately 30d during the hot seasons, and 40d in cold seasons. The experimental periods were chosen based on the rotational grazing management plan of the herd (Sharpe et al., 2020) associated of typical days of each climatic season, avoiding rainy and cloudy days.

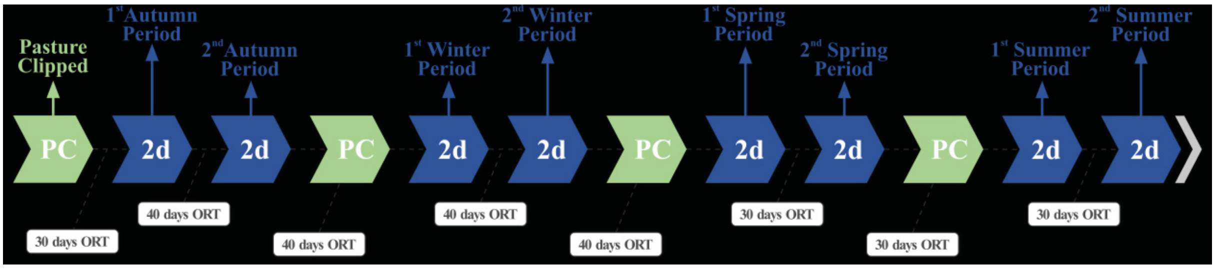

Figure 32-1 Schematic representation of the experimental periods in relation to days of optimum recovery time (ORT) by season after the pasture clipped (PC).

Cloudless days were a requirement to measure the intensity of animals' use of the different areas of silvopastoral systems. The selection of the experimental days was based on multiple weather forecasts, assuming a longer experimental period as a security margin. This ensured that the experimental days had the same weather characteristics (cloudless and without rain) as required to carry out the experiment in relatively homogeneous conditions but in an uncontrolled environment. The focus of the study was not to characterize the microclimate of the season but the effect of the microclimate on the behavior of the animals. The duration of data collection was based on the methodology used by de Sousa et al. (2021b); Deniz et al. (2020, 2019); Giro et al. (2019); Stivanin et al. (2019); Vizzotto et al., (2015); and Volpi et al. (2021).

32.4 MEASUREMENTS

The data collection was performed between the morning and the afternoon milking (8:00 to 15:00); during this period, microclimatic variables and cows' behavior were measured.

32.4.1 Microclimate and Bioclimatic indicators

Local microclimatic variables measured were air temperature (AT, °C), relative humidity (RH, %), wind speed (WS, m/s), soil surface temperature (SST, °C) and black globe temperature (BGT, °C). The measurements were taken with a 5-min interval from 8:00 to 15:00, simultaneously with behavioral observations. This interval between the morning and the afternoon milking was chosen because the lactating group was moved into a new paddock after each milking. Air temperature and relative humidity were measured [inside at the meteorological shelter (ISO 7726, 1998)] by a DHT22 sensor (AT: -40ºC to 80ºC scale, ± 0.5 °C precision; and 0.1°C resolution; RH: 0% to 100% scale, ± 2% precision; 0.1% resolution). The wind speed was measured using a thermo-anemometer (Model HM-833; 0.1 to 35 m/s scale; ± 7% + 0.70 m/s precision; 0.01 m/s resolution). The soil surface temperature was measured by a the DS18b20 thermal sensor (-55ºC to 125ºC scale, ± 0.5°C precision; and 0.1°C resolution). The black globe temperature was measured by a DS18b20 thermal sensor fixed at the center of a black globe [black hollow sphere (Vega et al., 2020)]. The set of sensors was coupled to a microcontroller (Arduino® Nano) and the data was stored on a micro SD card (de Sousa et al., 2021ab). In the silvopastoral systems two sets of sensors were located at full sun exposure (sunny condition - distant from the trees), and at the shaded condition, 2m distance from the trees (Figure 32-2).

Figure 32-2 Schematic representation of sampling points (A - shaded areas and B - sunny areas) in the silvopastoral system.

Due to the movement of shade throughout the day, when necessary, the set of sensors were moved to ensure they remained in their respective conditions (shaded and sunny; Karvatte et al. 2016; Deniz et al. 2019). The variables AT, RH, WS and BGT were measured at a height of 1.3m from the ground, which corresponded to the height of the mass center of an adult Jersey cow; the SST was measured at the soil below the pasture. The meteorological data from the experimental region were collected daily by an autonomous meteorological station (station ID - 25254905) belonging to SIMEPAR (in Portuguese – Sistema de Tecnologia e Monitoramento Ambiental do Paraná) located in "Piraquara" – PR (25º26'30"S, 49º03'48"W), 10km far from the experimental area.

With the microclimatic data, we determined the following bioclimatic indicators: black globe-humidity index (BGHI), and radiant heat load (RHL). The BGHI was calculated as proposed by Buffington et al. (1981), using the equation 2. The values obtained indicate: ≤74: thermal comfort situation; 75–78: warning; 79–84: danger; and ≥85: emergency (Baêta and Souza, 2010).

$$BGHI = BGT + 0.36 (DPT) + 41.5 \qquad (2)$$

Where: BGHI is the black globe-humidity index; BGT is the black globe temperature (°C); and DPT is the dew point temperature (°C).

The dew point temperature (DPT) was calculated by equation 3 developed by Wilhelm (1976).

$$DPT = (AT – (100 – RH)/ 5) \quad (3)$$

Where: DPT is the dew point temperature (°C); AT is the air temperature (°C), and RH is the relative humidity (%).

The RHL was used to express the total radiation received directly and indirectly by the animals, obtained by equation 4 proposed by Esmay (1982).

$$RHL = \sigma \times (T_m^2) \quad (4)$$

Where: σ is the Stefan-Boltzman constant, 5.67×10^{-8} K4 (W/m^2); and T_m is the mean radiant temperature (W/m^2).

32.4.2 Animals

All lactating cows at the farm participated in this study, as they already formed a stable social group. Furthermore, given that our experiment was conducted on commercial organic farm, the experiment had to be included in the daily routine of the farm. A total of 39 Jersey cows (Bos taurus taurus) participated in this study. The cows had similar light brown coat color, 5.2 ± 2.5 (mean ± SD) years old, average weight of 396.6 ± 42kg, milk production of 16.8 ± 3.6 L/day, and with average days in milk 144.4 ± 94.4 (details are shown in the supplementary Table S3). The group size was kept constant at 22 cows per season, but group composition was dynamic throughout the year, with cows entering the lactating group after calving and leaving approximately

60d before calving. However, it is important to emphasize that cows were moved in and out of the lactating group only during the intervals between the experimental periods; i.e., during each season the social group was stable. All cows had previous experience with the SPS since early life, as well as with the presence of observers. The animals remained in the experimental paddocks during the data collection (from 8:00 to 15:00) and, after this period, all cows were moved to milking. During the time between experimental periods (30d during the hot seasons, and 40d in cold season) the cows occupied other paddocks at the farm to allow the regrowth of pasture on the experimental paddocks.

32.4.2.1 Social rank determination

Observations of aggressive interactions were carried out twice per season, one week before each experimental period in the SPS. For each season, after morning milking, aggressive interactions (displacements) at the feeding area were observed for 5 consecutive days and lasted for 1h, starting after morning milking (approximately at 8:00). The feeding area was already known by the animals and had a rectangular shape with approximately 300 m2, concrete floor, and wooden fences. The feeder (40 m long) was covered with fiber–cement tiles (3 m high). The duration of social observation was based on the methodology used by Foris et al. (2018), McDonald et al. (2020), Vargas-Bello-Pérez et al. (2020). During these periods all displacements were registered through the methodology used by Deniz et al. (2021).

All displacements with physical contacts (Kondo and Hurnik, 1990) at the feeder were recorded continuously, whenever they occurred. We considered a displacement when a butt or a push from the actor (animal initiating the interaction) resulted in the complete withdrawal of the reactor’s (animal losing the interaction) head from the feed rail. After collection, the data were analyzed based on the proposed calculations by Kondo and Hurnik (1990), with the aid of the ETlog software (Deniz, 2018), to determine the dominance value. The ETlog builds a sociometric matrix with the number of wins and losses in the displacements of each animal relative to every other animal in the group. With the sociometric matrix, the ETlog software calculated the linearity index of the herd based on Landau (1951), applied the improved test of linearity (h’) due to the unknown relationships as described by de Vries (1995). Social hierarchy was then assigned according to dominance value, as described by (Coimbra et al., 2012). As in this study, we chose to divide the animals into three social categories [dominant (D), intermediate (I) and subordinate (S)], adopting the divisor 3. Social hierarchy (HS) was estimated by the distance between the highest (+X) and the lowest (−Y) dominance value, plus 1 (corresponds to the dominance value zero), which determines the number of points in the range.

32.4.2.2 Cows’ location and behavior

To evaluate the cows' location, we recorded in which area (shaded or sunny) the animals were when performing each behavior. A cow was considered using the shaded area when she had more than 50% of her body in the shade of tree; and a cow was considered using the sunny area when she had more than 50% of her body in the sun (Deniz et al., 2020; Giro et al., 2019).

Each cow was identified with a number painted on the lumbar area with commercial animal marking crayon. The posture (standing and lying) and behaviors grazing, idling, rumination, and other behaviors (defined by Coimbra et al. 2012, and Agudelo et al. 2013) were directly recorded by 10-minute interval scan-sampling; drinking water was recorded continuously whenever it occurred (Altmann, 1974; Lehner, 1996). Definitions of the behaviors are shown in supplementary Table S4. All behavioral observations were made by two previously trained researchers. Inter-observer reliability test (Lehner, 1996) was performed among the two researchers before the beginning of the data collection, with an agreement of 94.3%.

32.5 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

All analyses [influence, descriptive (frequency, average, standard deviation, coefficient of variation, minimum and maximum), and confirmatory] were performed through the statistical software R version 4.0.5 (R Core Team 2021). The database was built with each observation [local microclimate variables (26,880 measurements), bioclimatic indicators (10,752 measurements) and frequency of behavior (88,704 observations)] synchronized by date and time of day, and the areas (shaded and sunny). The descriptive analysis was based on the data summary by areas (shaded and sunny), since they were all observed for the same period of time (7h/ d). The models were adjusted through the maximum likelihood-Laplace approximation method in the statistical package lme4 (Bates et al., 2015). The confidence intervals were estimated using Type II Wald chi-square tests and the fit of the model was given by a likelihood-test. The fitness of the models was tested by inspecting the residual in the graphs, a line of best fit. The normality of the random facts was given by quartile plot means with a confidence interval of 95%. The seasons were considered as replicated experiments. The details of each statistical model performed for microclimate, bioclimatic indicators, and behaviors are describe below.

32.5.1 Analysis of the microclimate and bioclimatic indicators

The experimental design consisted of two repetitions by experimental period, two independent variables (shaded and sunny areas) and seven dependent variables (AT, RH, WS, SST, BGT, BGHI, and RHL) measured over time (8:00 to 15:00). To confirm that areas (shaded and sunny), influenced on microclimate variables and the thermal comfort indexes, the data were analyzed using a mixed model (Generalized Linear Models - GLM) at 95% confidence level, following the model:

$$Y_{ij} = \alpha_j + \beta_{ij} + e_{ij}$$

Where: Yij are the microclimatic variables or thermal comfort index; αj are the fixed effects of the areas (shaded and sunny) provided by the SPS; βij is the random effect, i corresponds to days; j corresponds to hours; and eij is the residual effect.

All analyzes were performed separately and each variable obtained a GLM model. In all GLM models, a Gamma distribution with a logarithmic link function was used. The data from the weather station (station ID - 25254905) was used for a general monthly descriptive analysis.

32.5.2 Analysis of cows' location and behaviors

To assess drinking water events in relation to the BGHI index and social hierarchy, the data were summarized by hour; number of events occurred in each hour. A generalized linear model with Poisson distribution was used and a confidence interval of 95%. The BGHI and social hierarchy were defined as fixed effects. For interpretation purposes, the incidence rate ratio (IRR) was used, and the predict events of drinking water and the BGHI were plotted in graphics.

In order to determine whether measures of cows' location had a relationship with the BGHI index and social hierarchy, a binomial regression model was performed. For the model, as the dependent variable was binary (dichotomous), we considered the Bernoulli distribution (Hardin and Hilbe, 2018) and tested the mains of bonding functions (Logit, Probit, and C.log-log) used in the binomial regression model (Pires and Diniz, 2012) to determine the most parsimonious model. The outcome of interest was the cows' location at the shaded or sunny areas (binary-dependent variable) associated with three independent variables (BGHI, social hierarchy, and animals' posture), following the model:

$$pLy_i = \frac{1}{1 + e^{-(\alpha + \beta_1 SH_i + \beta_2 BGHI_i + \beta_3 animals' posture_i)}}$$

Where: pLy_i is the probability of the outcome occurring for each observation *i*; α is the intercept of the logistic model; β_1, β_2, and β_3 are the estimated parameters for each independent variable. For interpretation purposes, the parameters are presented in Odds Ratio (OR). This is expressed as OR= $e^{\beta 1}$, OR= $e^{\beta 2}$ and OR= $e^{\beta 3}$. In order to make the model more robust, the BGHI (≤74: thermal comfort; 75–78: warning; and 79–84: danger; Baêta and Souza, 2010), social hierarchy (dominant, intermediate, and subordinate; Coimbra et al., 2012), and animals' posture (lie down and standing) were grouped into classes.

To confirm that the areas (shaded and sunny) influenced on the behavior of the cows, the data were analyzed by a mixed GLM with Poisson distribution and 95% confidence interval. The experimental design consisted of two repetitions by experimental period, animals as sample units, two independent variables (shaded and sunny conditions) and the dependent variables were each behavior measured over time (8:00 to 15:00) and summarized by hour. As the “other behaviors” occurred in a low frequency, we did not include it in the statistical analyzes. The areas were defined as fixed effects (shaded was the reference area), and the days and hours were defined as random effects. For interpretation purposes, the IRR and the frequency of the behavior measured by each area were used.

To measure the relationship between the areas (shaded and sunny), social hierarchy (dominant, intermediate, and subordinate), BGHI index (thermal comfort, warning, and danger), and seasons (autumn, winter, spring, and summer) with the cows' behavior, we built a GLM model for each behavior. In order to make the model more robust, for the lying behaviors we included the soil surface temperature as a continuous variable (de Sousa et al. 2021a). For the model interpretation purposes the IRR was used. In addition, the frequency of behaviors was submitted to confirmatory analysis by the Wilcoxon-Mann-Whitney test of independent samples, (95% of confidence level) to see if had a difference in the time spent by each social category in the shaded areas. The generalized linear model with Poisson distribution (95% of confidence level) used to measure the associations was:

$$Y_{ij} = A_i + H_i + \alpha_{ijk} + \beta_{ij} + e_{ij}$$

Where: Yij is the behavior measured; Ai are the fixed effects of areas; Hi is the fixed effect of social hierarchy; αij are the fixed effects, i corresponds to the BGHI index, j corresponds to seasons, k corresponds to the soil surface temperature for lying behaviors; βij is the random effect, i corresponds to days, j corresponds to hours, and eij is the residual effect.

33 RESULTS

33.1 MICROCLIMATIC AND BIOCLIMATIC INDICATORS

The monthly variation of environmental variables for the experimental region is shown in Figure 33-1. The values of the microclimatic variables differed according to the area (shaded and sunny) and hours of the day (Supplementary Table S5).

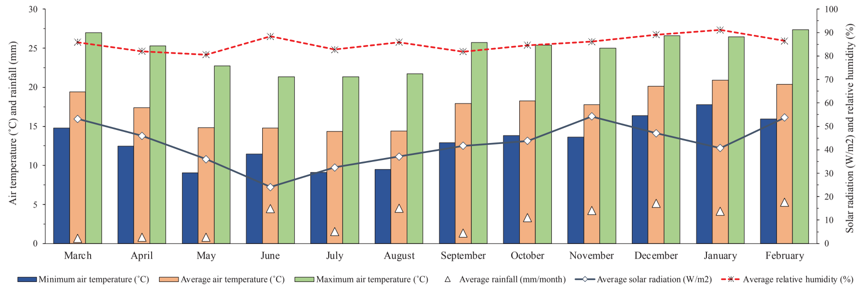

Figure 33-1 Climate monthly average in the experimental environment. Records collected daily by the autonomous meteorological station (ID – 25254905) belonging to SIMEPAR.

In the shaded areas, the tree canopy provided on average a thermal reduction of 23% in cold seasons and 26% in hot seasons. In addition, the shaded areas had lower average values of microclimatic variables than the sunny areas for all variables ($p<0.05$), except for relative humidity (supplementary Table S6). In all seasons, the sunny areas promoted potential thermal discomfort for the cows (BGHI above 74) and the critical period for RHL was in the afternoon.

33.2 COWS' LOCATION AND BEHAVIORS

33.2.1 Relationship between BGHI index and social hierarchy with drinking water events

The higher number of drinking water events was in the summer (319 events), followed by spring (239 events), autumn (184 events), and winter (164 events). For all social categories, the odds of drinking water decreased ($p<0.001$), for each increase in BGHI unit (autumn: 7%, winter: 8%, spring: 5%, and summer: 2%). Dominant cows were less likely (IRR = ~50%; $p<0.001$) to drink water than intermediate and subordinate cows (Figure 33-2). Intermediate (I) and subordinate (S) cows were more likely to drink water ($p<0.001$) than dominant cows (IRR = autumn: I - 55%, S – 8%; winter: I - 80%, S – 70%; spring: I - 54%, S – 74% and summer: I - 15% S – 16%).

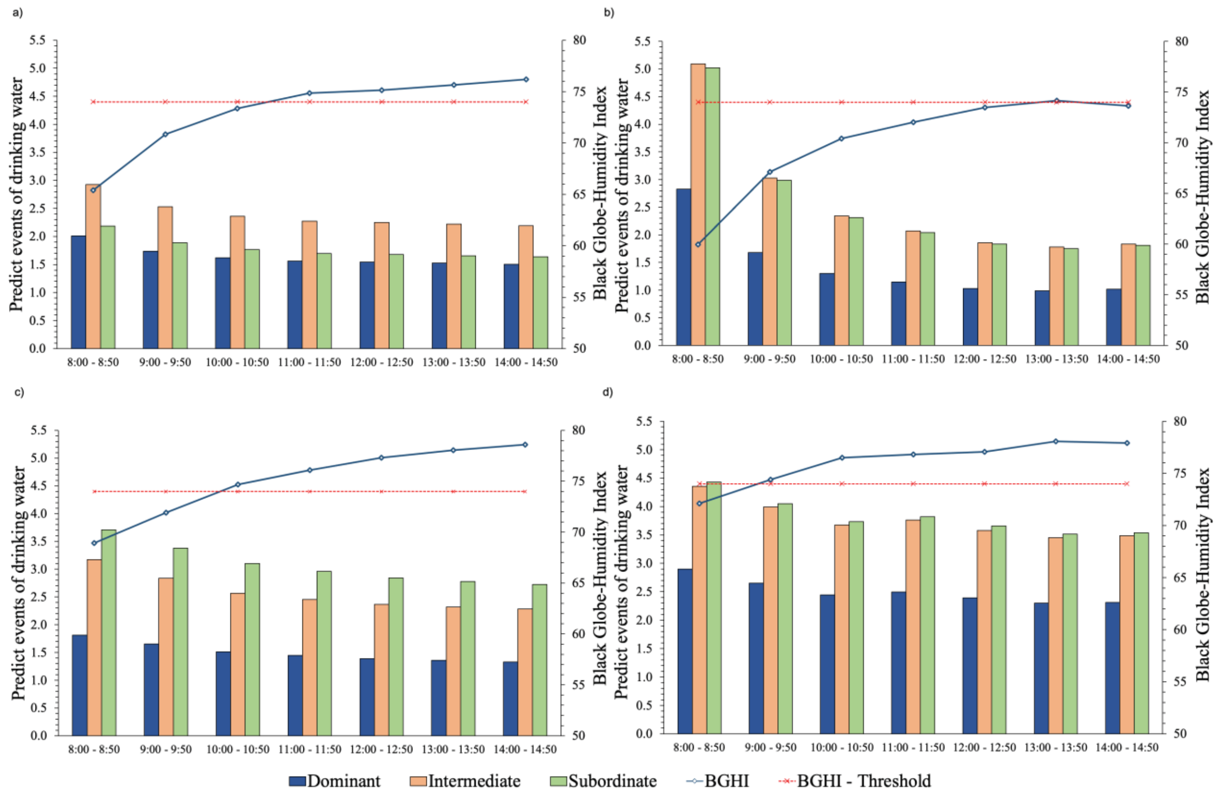

Figure 33-2 Predicted events of drinking water by social categories (dominant, intermediate and subordinate) in relation to average of the black globe-humidity index (BGHI) by season (a – autumn, b – winter, c – spring, and d – summer). Values represent the predicted means of the drinking water events for all cows in each social category.

33.2.2 Relationship between cows' location, BGHI index, and social hierarchy with lying behavior

The details from the binomial regression model built to determine the relationship between the cows' location (shaded and sunny), thermal environment (BGHI), and social hierarchy with the animals' posture are shown in Table 33-1. In general, the odds of a cow lying in the sunny areas was 62% lower than in the shaded areas ($p<0.001$). The odds of a cow using the sunny areas were 46% higher among cows of intermediate than dominant rank ($p<0.001$) and 2.1 times higher among cows of subordinate than dominant rank ($p<0.001$).

Table 33-1 Posterior estimates of the binomial regression model with the Bernoulli distribution, logit link function, and 95% of confidence intervals (CI) for areas of the silvopastoral system (SPS), social hierarchy, black globe-humidity index, and animals' posture.

Predictor	Parameter	Odds ratio	CI		z value	p-value
			Lower	Upper		
Areas of the SPS	Dependent variable	0.280	0.252	0.310	-24.41	<0.001
Social hierarchy	Dominant	Ref.	-	-	-	-
	Intermediate	1.46	1.29	1.64	6.36	<0.001
	Subordinate	2.11	1.87	2.38	12.20	<0.001
Black globe-humidity index	Thermal comfort (≤74)	Ref.	-	-	-	-
	Warning (range: 75 to 78)	0.87	11.89	14.74	47.33	<0.001
	Danger (range: 79 to 84)	0.66	29.46	39.91	45.64	<0.001
Animals' posture	Lying down	Ref.	-	-	-	-
	Standing	0.38	0.34	0.44	-15.24	<0.001

33.2.3 Influence of the cows' location on the time spent in each behavior

The time cows spent performing each behavior in the different locations of the silvopastoral system is shown in Table 33-2. Cows spent more time performing comfort behaviors (idling and rumination lying) in the shaded than in the sunny areas ($p<0.05$) in autumn and summer. In contrast, in the winter cows were 75% less likely to perform comfort behaviors (idling and rumination lying) in the shaded than in the sunny areas ($p<0.01$).

Table 33-2 Effect of cows' location [shaded (ref.) and sunny] on the frequency (%) of behaviors in the four seasons.

Autumn				
Behaviors	Shaded		Sunny	p-value
	Freq. (%)	IRR	Freq.	
Grazing	13.8	0.2	41.7	<0.001
Standing idling	8.1	6.2	1.3	<0.001
Standing rumination	8.8	8.8	1.0	<0.001
Lying idling	7.3	2.6	2.9	<0.001
Lying rumination	10.9	3.0	3.7	<0.001
Others	0.4	-	0.1	-

Winter				
Behaviors	Shaded		Sunny	p-value
	Freq. (%)	IRR	Freq.	
Grazing	11.1	0.2	47.4	<0.001
Standing idling	3.8	1.7	2.3	<0.001
Standing rumination	5.8	1.5	3.8	<0.001
Lying idling	3.9	0.7	5.8	<0.001
Lying rumination	6.7	0.9	7.8	0.1
Others	0.5	-	1.1	-

Spring				
Behaviors	Shaded		Sunny	p-value
	Freq. (%)	IRR	Freq.	
Grazing	13.8	0.3	45.7	<0.001
Standing idling	5.1	2.8	1.8	<0.001
Standing rumination	11.7	2.5	4.8	<0.001
Lying idling	2.1	1.0	2.2	0.876
Lying rumination	4.8	1.1	4.2	0.223
Others	0.8	-	2.8	-

Summer				
Behaviors	Shaded		Sunny	p-value
	Freq. (%)	IRR	Freq.	
Grazing	16.0	0.4	38.1	<0.001
Standing idling	18.3	6.0	3.1	<0.001
Standing rumination	9.7	3.2	3.0	<0.001
Lying idling	2.6	2.0	1.3	<0.001
Lying rumination	3.6	5.8	0.6	<0.001
Others	1.4	-	2.2	-

Frequency (Freq., %) followed by p-value. The incidence rate ratio (IRR) represents the odds of a given events occurring in relation to the reference category (shaded areas).

33.2.4 Relationship between the cows' behavior and location (in the shaded or sunny areas), social hierarchy, BGHI, seasons, and soil surface temperature

The details from the multilevel linear regression model built to determine the relationship between the cows' location (shaded and sunny), thermal environment (BGHI), and the social hierarchy with the cows' behavior are shown in supplementary Table S7. The highest odds of standing behaviors (idling and rumination) occurred in the hot seasons, compared to autumn ($p<0.001$). At the danger category of BGHI, the cows were twice as likely to be standing idling as under the thermal comfort category of BGHI ($p=0.02$). The cows were more likely (1.46 time) to be standing ruminating at the warning category than at the thermal comfort category of BGHI ($p<0.001$).

Grazing was the most performed behavior regardless of the season, and it was approximately 5 times more likely ($p<0.001$) to occur when the cows were located in the sunny area (supplementary Table S7). The odds of grazing were 11% lower at the warning than at the thermal comfort category of BGHI ($p<0.001$). Conversely, cows were 19% less likely to perform grazing behavior at the danger BGHI than at thermal comfort ($p<0.001$).

The soil surface temperature influenced cows' location when lying ($p<0.001$); for each additional SST unit, lying behavior increased by 9% (supplementary Table S7). The time spent in each behavior differed among the social categories in the shaded areas ($p<0.05$; Table 33-3). Dominant cows spent more time lying in the shaded areas than intermediate and subordinate cows ($p<0.001$), except in the winter, when the odds of cows in this category lying ruminating in the sunny areas were 90% higher than in the shaded areas ($p<0.01$).

Table 33-3 Frequency (%) of behaviors according to the cows' social hierarchy [dominant (ref.), intermediate, and subordinate], in the shaded areas of the silvopastoral system in the four seasons.

Autumn			
Behaviors (%)	Dominant	Intermediate	Subordinate
	Freq.	Freq.	Freq.
Grazing	26.6^{b}	33.3^{a}	21.9^{b}
Standing idling	13.3^{a}	16.9^{a}	20.7^{a}
Standing rumination	17.1^{b}	17.2^{b}	20.4^{a}
Lying idling	17.9^{a}	12.1^{c}	15.4^{b}
Lying rumination	25.0^{a}	20.5^{b}	21.6^{b}
Total freq.	100.0	100.0	100.0

Winter			
Behaviors (%)	Dominant	Intermediate	Subordinate
	Freq.	Freq.	Freq.
Grazing	29.2^{c}	34.1^{b}	42.6^{a}
Standing idling	15.8^{a}	9.8^{c}	12.5^{b}
Standing rumination	23.4^{a}	16.5^{b}	16.7^{b}
Lying idling	11.7^{a}	13.5^{a}	11.9^{a}
Lying rumination	19.9^{b}	26.1^{a}	16.4^{b}
Total freq.	100.0	100.0	100.0

Spring			
Behaviors (%)	Dominant	Intermediate	Subordinate
	Freq.	Freq.	Freq.
Grazing	34.0^{c}	38.3^{a}	37.3^{b}
Standing idling	9.5^{b}	15.6^{a}	14.8^{a}
Standing rumination	26.1^{c}	30.0^{b}	39.7^{a}
Lying idling	9.0^{a}	4.9^{b}	2.7^{c}
Lying rumination	21.4^{a}	11.1^{b}	5.5^{c}
Total freq.	100.0	100.0	100.0

Summer			
Behaviors (%)	Dominant	Intermediate	Subordinate
	Freq.	Freq.	Freq.
Grazing	28.8^{b}	32.6^{a}	33.1^{a}
Standing idling	26.1^{b}	39.7^{a}	40.4^{a}
Standing rumination	15.8^{b}	21.1^{a}	19.9^{a}
Lying idling	11.4^{a}	3.4^{b}	2.6^{c}
Lying rumination	17.9^{a}	3.2^{b}	4.0^{b}
Total freq.	100.0	100.0	100.0

Frequency (freq., %) followed by the same letter in the line do not differ ($p<0.05$).

34 DISCUSSION

The cows' location in the silvopastoral system was influenced by the black globe-humidity index and the social hierarchy; in addition, soil surface temperature influenced the lying behaviors. Challenging thermal conditions in the seasons with higher black globe-humidity index increased the animals' motivation to seek shade. However, the social position within the herd influenced the cows' location within the system, with dominant cows spending more time in shaded areas regardless of the season. This may be related to the body size of dominant cows, which are generally heavier (Deniz et al., 2021; Šárová et al., 2016); and increased body size reduces the metabolic rate per unit surface area to achieve thermal equilibrium. Possibly to compensate for the lower access to shade, intermediate and subordinate cows were more likely to drink water than dominant cows in all seasons. Our findings indicate that the cows' use of shade is associated with the need to keep thermal balance, but the strategies to deal with the thermal challenges are affected by the social hierarchy. Regrouping, a common management in dairy farms (Smid et al., 2019; Walker et al., 2015), may affect the established social hierarchy (Hubbard et al., 2021). To minimize this problem, the groups were kept stable during the experimental periods; cows were moved in and out of the lactating group only between experimental periods, and we determined the social hierarchy the week before each experimental period.

The influence of social hierarchy on the time that cows spent in the shaded area was less noticeable in spring and summer than in the colder seasons. In the hotter seasons, due to the challenges posed by the thermal environment, subordinate cows were more motivated to access the shade, but remained standing. The value an animal gives to a particular resource is dependent not only on the quality of the resource, but on its availability and needs of the animal. Thus, during the hot season, higher thermal stress would increase the need for shade and, consequently, subordinate cows would pay the cost of facing a dominant cow to use the shade. The motivation to access shade may be associated to intrinsic and extrinsic factors that increase linearly with increasing thermal discomfort. Intrinsic factors include physiological responses to heat stress such as rectal temperature, respiratory rate, heart rate (Skonieski et al., 2021) and, as shown here, social behavior. Extrinsic factors are those related to the thermal environment, such as solar radiation, air temperature, and relative humidity (Volpi et al., 2021; Magalhães et al., 2020). Thus, when an animal is motivated to change its environment, for example to alleviate the effect of thermal stress, it will initiate a reward cycle, where the strongest positive affective state occurs when the reward is acquired (e.g., when its rectal temperature decreases; Skonieski et al., 2021). However, preventing animals from performing these motivated behaviors, either by resource limitations or by their social position, can result in stress responses (Dawkins, 1988; Jensen and Pedersen, 2008). We can suggest that for subordinate cows the reward to access shade during the hot seasons may be greater than the challenge posed by their position in the social hierarchy.

Although subordinate cows remained longer in the shaded areas during hot than during the cold seasons, they were less likely to lie in the shade than dominant cows. One possible explanation is that the social stress of being subordinate may cause cows to be more vigilant. In contrast, the proximity of subordinate cows may allow dominant cows to relax during the idling period and reduce their vigilant behavior (Gygax et al., 2010). It is already known that lying down is an important behavioral indicator of welfare (Tucker et al., 2020), which can be influenced by environmental factors such as precipitation, solar radiation, air velocity (Tullo et al., 2019), and soil surface temperature (de Sousa et al., 2021a). In the summer, the lower soil surface temperature in the shaded areas may have promoted a comfortable surface for cows to lie down, given that lying on cooler surfaces favors heat exchanges by conduction (Dimov et al., 2017; Nordlund et al., 2019). In contrast, in winter the cows possibly spent more time lying in sunny areas in an attempt to gain heat. It is possible that due the great thermal amplitude between day and night in the winter (de Sousa et al., 2021b), dairy cows prefer to spend more time lying down in sunny areas (de Sousa et al., 2021a). Heat exchange (gain or loss) by conduction is a recent research topic in dairy cattle, but studies have focused on evaluating the issue in confined systems (Gebremedhin et al., 2016; Nordlund et al., 2019). In these systems, efforts have been made to find bedding materials (e.g., mattress, woodchip, and sand concrete) that are comfortable for animals and have good conductivity (Dimov et al., 2017; Nordlund et al., 2019; Schütz et al., 2020; Tucker et al., 2020). However, the effects of soil surface temperature and precipitation on heat exchange trough lying behavior of dairy cows raised on pasture has been explored in fewer studies (de Sousa et al., 2021a; Thompson et al., 2019). This highlights the need for greater research effort on the effects of these factors on the thermal balance of animals on pasture.

Our study shows that social hierarchy influences the behavioral strategies cows use to cope with thermal challenges. Interestingly, cows of different social categories adopted different strategies to alleviate their thermal discomfort; while dominant cows were more likely to remain longer in the shaded areas, intermediate and subordinate cows were more likely to drink water. Social dominance exerts an important influence of the access of animals to resources; animals at the top of the social hierarchy are usually the first to access a food resource (de Sousa et al., 2021c) and occupy an advantageous spatial position compared to others in the herd (di Virgilio and Morales, 2016). In free-stalls, cows increase the frequency of visits to the water trough and their water intake when the thermal environment becomes more challenging, but subordinate cows avoid visiting the water trough in the hottest and most competitive hours (McDonald et al., 2020). In compost-barn, primiparous cows, which are usually subordinate, are more likely to drink water in the hottest hours, while multiparous cows, which are usually dominant, are more likely to lie down in ventilated areas during the hottest hours of the day (Vieira et al., 2021). This emphasizes that the adaptive strategies to mitigate the effects of heat stress adopted by dairy cows can be influenced by the social category. For example, some of the adaptive measures cows raised on pasture use to mitigate heat stress are to increase standing time in the shaded areas (Deniz et al., 2020) and night grazing (Skonieski et al., 2021). Likewise, in a water restriction situation, while dominant non-lactating cows drank water every day, subordinate non-lactating cows drank water every other day; however, subordinate lactating cows would fight to drink daily, possibly because their physiological state increased their motivation to drink (Hötzel et al, 2013). It is thus possible that in our study the dominant cows compensated the water intake during the hours of lower thermal challenge; unfortunately, it was not possible to register cows' behavior during the night.

Although our study had a shaded area of 10.7 m2/cow, larger than the recommended for dairy cattle (2m^2/animal, Stivanin et al., 2019; 3.5m2/animal, Buffington et al., 1983; 5.6m2/animal, Collier et al., 2006), subordinate cows were less likely to use shade than dominant and intermediate cows. Even having shade space available to allow the subordinate cows to remain far from the dominant ones, the subordinate cows remained close to the rest of the group but in the sunny areas. This behavior, called grouping, is an effective strategy to mitigate predation risk and has been observed in domestic livestock (Grant and Albright, 2001). During feeding, subordinate cows avoid dominants when they have the opportunity (Bica et al., 2020; Rioja-Lang et al., 2012). In our study, the dominant cows used the shaded area to perform comfort behaviors (idling and rumination lying down), whereas the subordinate cows performed these behaviors in the sunny areas. In small and stable groups (as was the case of this study) social learning and social facilitation cause animals to synchronize their behavior, such as lying down (Stoye et al., 2012) and feeding (Raussi et al., 2011; Rook and Huckle, 1995). Further studies should explore the influence of social facilitation on the animals' access to areas that provide greater thermal comfort. Another interesting point that should be addressed in future studies is the influence of different tree arrangements (e.g., multiples rows, scattered trees, and wood systems) on the social behavior. Previous studies found effect of different tree arrangements on cattle behavior (de Sousa et al., 2021b; Domiciano et al., 2018; Oliveira et al., 2021) and this may affect shade use by different social categories. Shade is an important resource for animals raised on pasture-based systems, and their motivation to use shaded areas is stronger in the summer (Cardoso et al., 2021). However, social hierarchy can influence resource use (e.g., shade) depending on availability; e.g., in the study of Cardoso et al. (2021) high ranked heifers spend more time in the shade under the trees plus cloth shade while lower ranking heifers spent most of their time under the simple tree shade.

Raising dairy cows in silvopastoral systems can provide a more comfortable environment for the animals and be a good alternative to improve the sustainability of livestock production. In silvopastoral systems, cows have the opportunity to choose different areas to fulfill their motivations, e.g., remaining in the sunny areas in cold seasons (winter) and spending more time in the shade in hot seasons (spring and summer). However, we would like to emphasize that the effects of social hierarchy occur even when there is plenty of shade area available for all animals to have simultaneous access. This is related to the natural behavior of cattle, which as prey animals tend to remain in groups to protect themselves from predators. Therefore, applying the knowledge of the social relationships among animals raised on silvopastoral systems may allow improving the use of shade areas by cows and then their welfare.

35 5. CONCLUSION

The shaded areas provided a more comfortable microclimate and thermal environment for dairy cows. The cows' location was influenced by the black globe-humidity index and social hierarchy; besides these factors, lying behavior also was influenced by the surface temperature of the soil. In the seasons that posed a higher thermal challenge the cows were more motivated to use the shaded areas. However, dominant cows had higher odds of using the shaded areas to idle and ruminate lying down than subordinate and intermediate cows; i.e., dominant cows were more likely to express their comfort behaviors in shaded areas.

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Conflict of Interest

The authors declare no conflicts of interest with respect to the research, authorship, and/or publication of this article.

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37 SUPPLEMENTARY FILE CHAPTER V

Supplementary Table 37-1 Mean values and interval of variation of temperature, relative humidity, and solar radiation from the nearest meteorological station during the experimental period.

Seasons	Months	Air temperature, °C		Relative humidity, %		Solar radiation, W/m^2	
		Average	Range	Average	Range	Average	Range
Autumn	March to June 2020	18.8	11.6 - 28.1	79.1	36.7 - 100	476.5	4 - 949
Winter	June to September 2020	17.3	8.5 - 25.2	78.5	35.7 - 100	377.5	2 -753
Spring	September to November 2020	19.1	10.1 - 34.3	70.4	23.6 - 100	532.1	12 - 1052
Summer	December 2020 to March 2021	19.3	13.4 - 27.5	79.1	32.7 - 98.7	559.7	24 - 1094

Supplementary Table 37-2 Mean percentage values of the crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and residue mineral (RM) per kg of dry matter in the shaded and sunny areas of the silvopastoral system in the cold (autumn and winter) and hot (spring and summer) seasons.

Parameters (%)	Cold seasons		Hot seasons	
	Shaded areas	Sunny areas	Shaded areas	Sunny areas
CP	15.2	16.9	20.7	18.6
NDF	63.8	63.8	64.5	64.2
ADF	28.5	27.4	30.2	30.4
RM	8.2	6.8	8.1	8.3

Supplementary Table 37-3 Details of age (years), weight (kg), milk production (L/day), days in milk (days), dominance value (DV), and social category (SC; D - dominant, I - intermediate and S - subordinate) of animals group in relation to the seasons.

Animal	Age	Weight	Milk production	Days in milk	DV	SC	Animal	Age	Weight	Milk production	Days in milk	DV	SC
Autumn													
1	3.20	344	12.4	40	-11	S	12	3.02	362	12.5	75	-17	S
2	4.63	398	18.1	120	-15	S	13	4.05	350	14.7	248	9	I
3	4.48	433	16.4	53	7	I	14	6.52	332	13.6	45	-3	I
4	6.05	479	14.5	41	-2	I	15	15.20	449	14.1	142	21	D
5	3.79	455	14.3	48	6	I	16	3.65	427	18.6	41	2	I
6	4.84	501	21.2	102	11	D	17	6.45	439	20.4	39	23	D
7	3.50	380	10.5	237	-4	S	18	3.69	331	21.6	42	-17	S
8	2.97	386	18.6	233	-13	S	19	8.4	412	15.8	27	0	I
9	5.76	380	19.2	49	-2	I	20	2.4	310	13.3	27	-8	S
10	5.74	471	18.3	67	19	D	21	10.2	386	12.5	24	-4	S
11	9.88	444	20.2	59	16	D	22	2.6	374	19.6	29	-8	S
Winter													
Animal	Age	Weight	Milk production	Days in milk	DV	SC	Animal	Age	Weight	Milk production	Days in milk	DV	SC
1	3.5	385	12.4	141	-3	S	16	3.9	427	15.8	142	3	I
2	4.9	397	12.8	221	-6	S	17	6.7	455	20.8	140	13	D
3	4.8	416	18.1	154	9	D	19	8.6	410	21.3	128	-1	I
5	4.1	428	18.4	149	4	I	20	2.7	344	14.6	128	-4	S
6	5.1	442	22.8	203	8	D	21	10.3	390	16.8	125	-4	S
9	6.0	361	18.8	150	-1	I	22	2.9	350	19.1	130	-1	I
10	6.0	469	19.2	168	16	D	23	6.2	397	23.3	78	2	I
11	10.2	404	24.4	160	15	D	24	4.3	350	21.3	98	3	I
12	3.3	347	12	176	-6	S	25	2.4	326	17.4	120	-6	S
14	6.8	361	13.6	146	-2	S	26	5.6	350	15.3	116	-1	I
15	11.5	465	21.3	243	16	D	27	2.7	347	17.4	83	3	I
Spring													
Animal	Age	Weight	Milk production	Days in milk	DV	SC	Animal	Age	Weight	Milk production	Days in milk	DV	SC
1	3.7	335.5	10.4	231	-10	S	20	2.9	340	9.7	218	-4	S
2	5.2	414	15.1	311	-5	S	22	3.1	401.5	18.1	220	-1	I
3	5.0	401.5	22.1	244	14	D	23	6.5	392	20.5	168	-4	S
5	4.3	426.5	21	239	8	D	24	4.5	368	22.1	188	3	I
7	4.0	380	23.4	69	-1	I	25	2.6	357	16.3	210	-9	S
9	6.3	359	21.6	240	3	I	26	5.8	392	14.1	206	1	I
11	10.4	427	23.1	250	10	D	27	3.0	422.5	17.9	173	4	I
12	3.5	361	13.9	266	-4	S	28	3.7	377	15.3	109	-8	S
14	7.0	362	13.6	236	-1	I	29	2.4	318	14.6	114	-7	S
16	4.2	398.5	15.4	232	5	I	30	4.2	415.5	18.7	88	-1	I
17	7.0	457	22.2	230	19	D	31	2.5	382.5	16.35	5	-12	S
Summer													
Animal	Age	Weight	Milk production	Days in milk	DV	SC	Animal	Age	Weight	Milk production	Days in milk	DV	SC
3	5.3	439	10.2	337	8	D	29	2.6	315	14.2	207	-14	S
5	4.6	421	14.6	332	5	D	30	4.4	404	10.35	181	0	I
7	4.3	404	18.7	162	1	I	31	2.8	421	16.6	51	-14	S
8	3.8	404	13.5	20	-13	S	32	2.8	415	14.5	80	1	I
9	6.5	368	14	333	0	I	33	5.3	411	11.16	157	4	I
11	10.7	465	16.8	343	8	D	34	9.1	439	11.75	54	-13	S
14	7.3	415	14.5	2	-1	I	35	7.6	465	19.9	8	5	I
17	7.2	427	20.1	323	16	D	36	3.8	404	19	17	-12	S
23	6.7	404	17.2	261	0	I	37	2.4	368	15.7	5	-12	S
25	2.9	380	15.6	303	-9	S	38	2.4	433	13.2	10	-13	S
27	3.2	365	12.8	266	3	I	39	3.1	380	17.8	23	-13	S

Supplementary Table 37-4 Definitions of posture and behaviors of cows.

Posture	Definition
Standing	Animal in a vertical position upright
Lying	Animal with the abdomen pressing against the ground
Behaviors	Definition
Grazing	Animal with the mouth below or at the level of the forage or grabbing forage, may be stationary or moving forward
Rumination	Animal chewing with lateral jaw movements with the head at the same level or above its body
Drinking water	Animal with the lips immersed in the water, with neck movements indicating water ingestion
Others*	Any other behavior not described above, like allogrooming, autogrooming and scratching
Idling	Animal still, not engaged in any of the behaviors described above

Behaviors defined based on Coimbra et al. (2012); *behaviors defined based on Agudelo et al. (2013).

Supplementary Table 37-5 Average values (AV) and coefficient of variation (CV) of the variables air temperature (AT), relative humidity (RH), wind speed (WS), soil surface temperature (SST), black globe temperature (BGT), black globe-humidity index (BGHI), and radiant heat load (RHL) on the different areas (shaded and sunny) of the silvopastoral system and seasons.

Autumn						Winter					
Variables	Areas of Silvopastoral system					Variables	Areas of Silvopastoral system				
	Shaded		Sunny		p-value		Shaded		Sunny		p-value
	AV	CV	AV	CV			AV	CV	AV	CV	
AT (°C)	21.7	0.16	25.4	0.14	<0.001	AT (°C)	19.6	0.23	22.9	0.19	<0.001
RH (%)	61.9	0.28	56.8	0.28	0.032	RH (%)	68.5	0.30	61.6	0.29	0.021
WS (m/ s)	1.0	0.68	1.4	0.64	0.139	WS (m/ s)	1.5	0.71	1.7	0.69	0.516
SST (°C)	19.3	0.09	27.2	0.24	<0.001	SST (°C)	16.6	0.16	22.3	0.27	<0.001
BGT (°C)	21.9	0.16	30.8	0.15	<0.001	BGT (°C)	19.7	0.24	27.9	0.19	<0.001
BGHI	68.1	0.04	78.0	0.06	<0.001	BGHI	65.6	0.08	74.6	0.08	<0.001
RHL	432.7	0.15	568.7	0.09	<0.001	RHL	420.3	0.07	551.3	0.12	<0.001
Spring						Summer					
Variables	Areas of Silvopastoral system					Variables	Areas of Silvopastoral system				
	Shaded		Sunny		p-value		Shaded		Sunny		p-value
	AV	CV	AV	CV			AV	CV	AV	CV	
AT (°C)	23.6	0.25	27.2	0.18	<0.001	AT (°C)	22.8	0.11	26.5	0.07	<0.001
RH (%)	52.5	0.31	48.0	0.24	<0.001	RH (%)	68.6	0.18	62.2	0.14	0.012
WS (m/ s)	1.5	0.39	2.1	0.39	0.248	WS (m/ s)	1.0	0.34	1.3	0.36	0.352
SST (°C)	21.4	0.25	36.6	0.22	<0.001	SST (°C)	23.4	0.13	31.5	0.18	<0.001
BGT (°C)	24.0	0.25	32.7	0.13	<0.001	BGT (°C)	25.8	0.12	33.0	0.08	<0.001
BGHI	70.3	0.10	79.9	0.06	<0.001	BGHI	71.0	0.07	81.0	0.03	<0.001
RHL	448.7	0.08	603.7	0.09	<0.001	RHL	453.7	0.08	620.0	0.06	<0.001

Averages values followed by the p<0.05 in the line did not differ.

Supplementary Table 37-6 Multilevel linear regression model of areas of SPS (shaded and sunny), social hierarchy (dominant, intermediate, and subordinate), black globe-humidity index (BGHI), soil surface temperature (SST - for lying behaviors), and seasons for each evaluated behavior.

Predictor	Parameter	IRR	CI		z value	p-value
			Lower	Upper		
Grazing						
Areas of the SPS	Shaded	Ref.	-	-	-	-
	Sunny	4.71	4.42	5.01	48.16	<0.001
Social hierarchy	Dominant	Ref.	-	-	-	-
	Intermediate	1.17	1.10	1.24	5.02	<0.001
	Subordinate	1.15	1.07	1.22	4.33	<0.001
Black globe-humidity index	Thermal comfort (≤74)	Ref.	-	-	-	-
	Warning (range: 75 to 78)	0.63	0.59	0.67	-15.00	<0.001
	Danger (range: 79 to 84)	0.48	0.45	0.52	-20.89	<0.001
Seasons	Autumn	Ref.	-	-	-	-
	Winter	0.92	0.85	0.98	-2.48	0.013
	Spring	1.06	0.99	1.13	1.77	0.076
	Summer	1.03	0.96	1.09	0.81	0.416
Standing resting						
Predictor	Parameter	IRR	CI Lower	CI Upper	z value	p-value
Areas of the SPS	Shaded	Ref.	-	-	-	-
	Sunny	0.16	0.13	0.19	-19,06	<0.001
Social hierarchy	Dominant	Ref.	-	-	-	-
	Intermediate	1.08	0.95	1.24	1.19	0.233
	Subordinate	0.99	0.87	1.15	-0.04	0.970
Black globe-humidity index	Thermal comfort (≤74)	Ref.	-	-	-	-
	Warning (range: 75 to 78)	1.53	1.21	1.93	8.52	<0.001
	Danger (range: 79 to 84)	2.27	1.12	4.61	2.29	0.022
Seasons	Autumn	Ref.	-	-	-	-
	Winter	0.64	0.53	0.76	-4.87	<0.001
	Spring	0.59	0.49	0.71	-5.53	<0.001
	Summer	2.13	1.85	2.44	10.59	<0.001
Standing rumination						
Predictor	Parameter	IRR	CI Lower	CI Upper	z value	p-value
Areas of the SPS	Shaded	Ref.	-	-	-	-
	Sunny	0.33	0.28	0.38	-14.91	<0.001
Social hierarchy	Dominant	Ref.	-	-	-	-
	Intermediate	0.90	0.79	1.02	-1.59	0.111
	Subordinate	1.03	0.91	1.17	0.48	0.635
Black globe-humidity index	Thermal comfort (≤74)	Ref.	-	-	-	-
	Warning (range: 75 to 78)	1.47	1.27	1.69	5.32	<0.001
	Danger (range: 79 to 84)	0.96	0.79	1.16	-0.40	0.689
Seasons	Autumn	Ref.	-	-	-	-
	Winter	0.92	0.79	1.08	-0.97	0.334
	Spring	1.59	1.37	1.84	6.16	<0.001
	Summer	1.26	1.09	1.46	3.06	0.002
Lying idling						
Predictor	Parameter	IRR	CI Lower	CI Upper	z value	p-value
Areas of the SPS	Shaded	Ref.	-	-	-	-
	Sunny	0.27	0.21	0.35	-10.23	<0.001
Social hierarchy	Dominant	Ref.	-	-	-	-
	Intermediate	0.61	0.52	0.71	-6.09	<0.001
	Subordinate	0.70	0.59	0.82	-4.34	<0.001
Black globe-humidity index	Thermal comfort (≤74)	Ref.	-	-	-	-
	Warning (range: 75 to 78)	1.77	1.38	2.27	4.54	<0.001
	Danger (range: 79 to 84)	0.79	0.56	1.10	-1.38	0.166

Soil surface temperature	Continuous	1.09	1.08	1.11	10.35	<0.001
Seasons	Autumn	Ref.	-	-	-	-
	Winter	1.11	0.94	1.32	1.26	0.207
	Spring	0.23	0.18	0.29	-12.21	<0.001
	Summer	0.24	0.19	0.29	-12.86	<0.001

Lying rumination

Predictor	Parameter	IRR	CI		z value	p-value
			Lower	Upper		
Areas of the SPS	Shaded	Ref.	-	-	-	-
	Sunny	0.31	0.26	0.38	-12.17	<0.001
Social hierarchy	Dominant	Ref.	-	-	-	-
	Intermediate	0.66	0.58	0.75	-6.53	<0.001
	Subordinate	0.59	0.52	0.68	-7.60	<0.001
Black globe-humidity index	Thermal comfort (≤74)	Ref.	-	-	-	-
	Warning (range: 75 to 78)	0.57	0.44	0.74	-4.25	<0.001
	Danger (range: 79 to 84)	0.19	0.98	0.38	-4.70	<0.001
Soil surface temperature	Continuous	1.09	1.08	1.11	13.54	<0.001
Seasons	Autumn	Ref.	-	-	-	-
	Winter	1.26	1.09	1.44	3.29	0.001
	Spring	0.40	0.34	0.48	-10.46	<0.001
	Summer	1.19	0.16	0.24	-16.34	<0.001

The incidence rate ratio (IRR) represents the events' odds that occurred, indicating how much a category influences the number of events in relation to the reference (Ref.) category.

38 GENERAL CONCLUSION

The ADEF proved to be an efficient and low-cost tool to measure environmental variables in livestock farming. With the ADEF aid it was possible to verify that the shaded areas provided a better thermal environment for dairy cows than sunny areas. Furthermore, the pattern found by data mining suggests that studies that evaluate the thermal comfort of dairy cows on pasture areas should use indices that consider solar radiation. The cows' location was influenced by the black globe-humidity index and social hierarchy. Dominant cows (older and heavier) remained longer time at the feeder and were more likely to use shaded areas in a silvopastoral system, while lower-ranking cows remained less time at the feeder and were more likely to drink water when remaining in a silvopastoral system. Thus, we advance in the knowledge of cows' thermodynamics raised in a silvopastoral system, through interdisciplinarity, integrating information from diurnal and social behavior of cows, data mining, and the use of accurate low-cost sensors to measure microclimatic variables.

39 FINAL CONSIDERATIONS

The silvopastoral system has the potential to provide comfortable areas (shaded) for dairy cows. To achieve improvements in pasture-based livestock, it is necessary for research to include evaluations in the animals, since that the benefits of silvopastoral systems can bring to animals' thermal comfort are well reported in the literature. However, the effects on the quality of life of animals, such as emotional states and the use of different thermoregulatory resources (shade and water) have been little explored. Thus, further research in silvopastoral system areas that relates the thermal environment and the social hierarchy, with thermoregulatory variables, is necessary to corroborate with the findings from this research and confirm the benefits of the silvopastoral system for dairy cows.

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ARTICLE

Age and body mass are more important than horns to determine the social position of dairy cows

Matheus Deniz[1] · Karolini Tenffen de Sousa[1] · Marcos Martinez do Vale[2] · João Ricardo Dittrich[1]

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Abstract
The aims of this observational study were to (1) define which animal's phenotypic characteristics determine social position in the context of a commercial organic farm with mixed herd (horned and non-horned cows) and (2) determine the influence of social position on the time at the feeder. We took the following measurements from 27 dairy cows in lactation: body mass, age, body condition score, body length, withers height, distance between horns, horn circumference and length. Replacement and time at the feeder were recorded for 1 h at the time of supplementation. Dominance values for each animal were calculated and the herd was divided into three social categories: dominant (D), intermediate (I) and subordinate (S). Age, body length and body mass influenced ($p < 0.001$) dominance value of all animals. The presence of horn influenced ($p = 0.034$) the dominance value of the I and S animals because it was a unique characteristic of these categories. Dominant (84.3%) and intermediate (75.2%) animals spend more time ($p < 0.05$) at the feeder than the subordinate (59.5%); however, dominant animals tended ($p = 0.093$) to spend more time at the feeder than the intermediate animals. The social position of an animal was influenced by its age, body mass and body length, and its social position influenced the time at the feeder.

Keywords Animal behavior · Applied ethology · Dominance value · Dyads level · Social hierarchy

Introduction

Social behavior plays an important role in an animal's life (Proudfoot and Habing 2015). To gregarious species, like bovines, social interactions are evolutionarily important to maintain their fitness relative to the environment in which they live (Mendl and Held 2001). Social position affects several behaviors, such as feed and water intake in groups (Coimbra et al. 2012; Bica et al. 2019a, b). As part of their repertoire of natural behavior, cows organize themselves into hierarchies according to their willingness and ability to fight for scarce resources. The social hierarchy is established through dominance relationships, which are defined on the basis of aggressive interactions between cows (Beilharz and Zeeb 1982; Kondo and Hurnik 1990). Characteristics, such as social learning, age, weight, and horns, are relevant to the animals` competitive capacity and, consequently, to their social position (Bouissou 1972; Šárová et al. 2013).

Presence of horn is a phenotypic characteristic of several breeds of dairy cattle. However, there is a general concern about horned cows in the herd, due to the injuries and stress that horned animals may cause to others (Waiblinger et al. 2001). Also, horned cows are not safe for farmers to handle (Knierim et al. 2015). Thus, a common procedure in dairy farms is the dehorning of animals. However, over the last few years, the growing public awareness of practices in livestock production systems leads to a demand to be closer to an animal's natural environment (Hötzel et al. 2017; Yunes et al. 2017). To meet consumer expectations, organic production is a good option. Organic production systems are known for their effort to keep their animals under species-appropriate conditions. It should be noted that the typical expectation is to leave the animal in its natural state with horns, and this alternative is already being applied in organic production since calf dehorning is prohibited (Brasil 2011). Therefore, organic dairy farms must be suitable for handling horned animals, as applying good animal husbandry to adapt facilities and improve management conditions, it may not

✉ Matheus Deniz
utfpr@hotmail.com

[1] Graduate Program on Animal Science, Federal University of Parana, Curitiba 80.035-050, Brazil

[2] Department of Animal Science, Federal University of Parana, Curitiba 80.035-050, Brazil

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ORIGINAL PAPER

Development and application of an autonomous data logger to measure environmental variables in livestock farming

M Deniz[1] · K T de Sousa[1] · I C Gomes[1] · J A Fabro[2] · M M do Vale[1] · J R Dittrich[1]

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Abstract
The environmental conditions of livestock farming exhibit a wide degree of variability. In this context, we developed the ADEF, an autonomous data logger to better understand the degree of environmental variables that farm animals are exposed. Each ADEF consists of a set of components: microcontroller, memory card, real-time clock module, ambient sensor (DHT22), two thermal sensors (DS18B20), and an external battery. To validate the accuracy of ADEF, two stages were performed: (1) evaluation in a controlled environment; and (2) evaluation in the field. In both validation, uncertainty analyses were performed in order to determine if a bias correction would be necessary. In the controlled environment, the ADEF recorded consistent data associated with low measurement uncertainty. The high and significant coefficient of determination (~0.9; $p<0.05$) between the ADEF and commercial data logger indicated statistical model quality and confirmed the accuracy of the measured data. In the field, a total of 40,100 measurements were used for subsequent analysis. Furthermore, the hourly variation in the ADEF variables showed the same pattern and a high correlation (~0.9) with the data from the nearest meteorological station. In the field, environmental variables measured by the ADEF demonstrated low hourly dispersion associated with low relative standard uncertainty. The performance of the ADEF system was satisfactory both controlled environment and field, demonstrating that the ADEF can be easily applied as a low-cost tool that allows a more efficient approach to measure the environmental variables in the field.

Keyword Big data · Low-cost devices · Microcontroller · Precision livestock farming · Thermal environment

Introduction

The environment is a determining factor for livestock, as it influences the productivity of the farm (Renaudeau et al. 2011, 2012) and the quality life of the animals (Shock et al. 2016). Due to climate change, the effects of the thermal environment will be increasingly intense, mainly due to increases in air temperature and the frequency of extreme weather events (Nidumolu et al. 2014); this could affect the availability of grain and pasture, and also the presence of pests and parasites (Gauly et al. 2013). This situation has promoted negative effects on production (Bohmanova et al. 2007; Hammami et al. 2013), fertility (Hansen 2009), and animal health (Sanker et al. 2013), in addition to increasing the risk of mortality (cows: Vitali et al. 2009; laying hens: Riquena et al. 2019). Thus, production systems that improve quality of life for farm animals are gaining attention in the scientific community (e.g., the reviews of Kadzere et al. 2002; Das et al. 2016; Dash et al. 2016; Polsky and von Keyserlingk 2017; Herbut et al. 2019).

The thermal environment is composed of air temperature, relative humidity, and solar radiation, and the intensity of these factors can cause thermal stress in farm animals. Thermal stress occurs when animals experience conditions outside their thermal comfort zone (Kadzere et al. 2002) and are unable to dissipate (or receive/produce) enough heat to maintain thermal balance. The thermal environment can affect animal performance immediately or have a delayed impact (St-Pierr4e et al. 2003; Herbut et al. 2018). Thus,

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M Deniz
matheus-utfpr@hotmail.com

[1] Laboratório de Inovações Tecnológicas em Zootecnia (LITEZ – UFPR), Departamento de Zootecnia, Universidade Federal do Paraná, Curitiba, PR, Brazil

[2] Laboratório Avançado de Sistemas Embarcados e Robótica (LASER), Universidade Tecnológica Federal do Paraná (UTFPR), Curitiba, PR, Brazil

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Research Article

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animal distribution; behavioural pattern; decision tree; pasture; precision livestock farming

Author for correspondence:
Matheus Deniz, Email: matheus-utfpr@hotmail.com

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Classification of environmental factors potentially motivating for dairy cows to access shade

Matheus Deniz[1,2], Karolini Tenffen de Sousa[1,2], Isabelle Cordova Gomes[2], Marcos Martinez do Vale[2] and João Ricardo Dittrich[1,2]

[1]Programa de Pós-Graduação em Zootecnia, Departamento de Zootecnia, Universidade Federal do Paraná, Curitiba, Brazil and [2]Laboratório de Inovações Tecnológicas em Zootecnia (LITEZ – UFPR), Departamento de Zootecnia, Universidade Federal do Paraná, Curitiba, Brazil

Abstract

The aim of this Research Communication was to apply the data mining technique to classify which environmental factors have the potential to motivate dairy cows to access natural shade. We defined two different areas at the silvopastoral system: shaded and sunny. Environmental factors and the frequency that dairy cows used each area were measured during four days, for 8 h each day. The shaded areas were the most used by dairy cows and presented the lowest mean values of all environmental factors. Solar radiation was the environmental factor with most potential to classify the dairy cow's decision to access shaded areas. Data mining is a machine learning technique with great potential to characterize the influence of the thermal environment in the cows' decision at the pasture.

The general public prefers production systems that promote heat abatement for farm animals, like shade on pasture and fans in indoor housing (Cardoso *et al*., 2018). However, heat stress is one of the main challenges of grazing cows, as animals on pasture are constantly submitted to great environmental variability. Nowadays, new approaches to data analysis (fuzzy logic, artificial neural networking and data mining) can help researchers interpret large databases, improving livestock farming through a better understanding of the production system. Pattern extraction by data mining potentially allows accurate decision-making. Data mining tasks have been used in several dairy production research areas in an attempt to detect problems such as dystocia and calving difficulty (Zaborski *et al*., 2017, 2018), mastitis (Sharifi *et al*., 2018) and factors affecting cow reactivity during milking (Neja *et al*., 2017). However, most such research has focused on confined animals and only a few papers have applied this technique to analysis of animal behaviour raised on pasture. Based on this, we hypothesized that data mining can be applied for rule extraction and to identify potentially motivating environmental factors for grazing dairy cows to access shade.

Material and methods

The experiment was conducted in accordance with guidelines laid down by the Animal Ethics Committee of the Universidade Federal do Paraná and national legislation.

Experimental area and management

The study was carried out on a commercial dairy farm in southern Brazil. Data collection was performed during summer (southern hemisphere). The experimental area had 4 paddocks (1.500 m^2/paddock), and each one was composed of a silvopastoral system (SPS). The silvopastoral system provided a total shaded area of 5 m^2/animal in each paddock and a sunny area of 33 m^2/animal. The cows were moved daily to a new paddock. The paddocks and SPS distribution were uniform, allowing us to evaluate one paddock per day.

Animals and frequency at the shaded and sunny areas

Lactating Jersey cows (n = 39), with similar coat colour (light brown), and weight (mean ± SD) of 450 ± 50 kg were observed during four days, for approximately 8 h each day (from 9 : 00 to 16 : 50). As the proposal of this study was to classify the cows' decision in relation to environmental factors regardless of behaviour, we evaluated the frequency of dairy cows located in different areas of the SPS. The frequency of animals in shaded and sunny areas was recorded by scan sampling at 10 min intervals. The cow was considered to be in the shaded area when

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43 SUPLEMENT D – MANUSCRIPT PUBLISHED IN THE APPLIED ANIMAL BEHAVIOR SCIENCE

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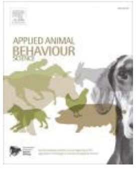

Social hierarchy influences dairy cows' use of shade in a silvopastoral system under intensive rotational grazing

Matheus Deniz [a,*,1], Karolini Tenffen de Sousa [a], Matheus Fernando Moro [a], Marcos Martinez do Vale [a,b], João Ricardo Dittrich [a,b], Luiz Carlos Pinheiro Machado Filho [c], Maria José Hötzel [c]

[a] *Laboratório de Inovações Tecnológicas em Zootecnia, Departamento de Zootecnia, Universidade Federal do Paraná, Curitiba 800035-050, Brazil*
[b] *Departamento de Zootecnia, Universidade Federal do Paraná, Curitiba 800035-050, Brazil*
[c] *Laboratório de Etologia Aplicada e Bem-estar Animal, Departamento de Zootecnia e Desenvolvimento Rural, Universidade Federal de Santa Catarina, Florianópolis 88034-001, Brazil*

A R T I C L E I N F O

Keywords:
Animal welfare
Applied ethology
Grazing dairy herd
Heat abatement
Social behavior
Thermal comfort

A B S T R A C T

The aim of this study was to evaluate the relationship between thermal comfort indicators and social hierarchy, and cows' location (shade or sun) and their diurnal behaviors in a silvopastoral system of a subtropical climate, covering the four seasons. We measured microclimatic variables (air temperature, relative humidity, wind speed, soil surface temperature) and cows' behaviors in two areas (shaded and sunny), as well as the influence of social hierarchy (dominant, intermediate, and subordinate) on cows' location (shade or sun). In addition, we determined the black globe-humidity index (BGHI) and radiant heat load (RHL) for both areas. Air temperature, wind speed, and soil surface temperature were lower in shaded areas, and relative humidity lower in the sunny areas ($p < 0.05$). The shaded areas provided on average a 23% RHL reduction in cold seasons (autumn and winter), and 26% in hot seasons (spring and summer). For cows of all social categories the odds of drinking water decreased ($p < 0.001$) for each additional BGHI unit increased. Dominant cows were less likely (~50%; $p < 0.001$) to drink water than intermediate and subordinate cows. In general, the odds of a cow lying in the sunny areas were 62% lower than in the shaded areas ($p < 0.001$). However, in winter cows were less likely (75%) to perform comfort behaviors (idling and rumination lying down) in shaded areas than in the sunny areas ($p < 0.01$). Furthermore, lying increased by 9% for each additional soil surface temperature unit. Dominant cows were more likely (~40%; $p < 0.001$) to lie down in the shaded areas than intermediate and subordinate cows. In conclusion, the cows' location in the silvopastoral system was influenced by the black globe-humidity index and social hierarchy; in a situation of higher thermal challenge cows were more motivated to use shade, but dominant cows were more likely to use the shaded areas than other cows.

1. Introduction

Conciliating trade-offs among livestock efficiency, maintenance of biodiversity, and ecosystem services will be the greatest challenges for the coming decades. Good health and animal welfare are considered essential to maintain low environmental impacts in the livestock sector (Broom, 2017). Raising animals in well managed pasture-based systems can contribute to the mitigation of climate change through soil organic carbon sequestration (Seó et al., 2017; Stanley et al., 2018), and improve their welfare as these systems allow animals to express their natural behaviors (Charlton and Rutter, 2017; Crump et al., 2021). However, heat abatement for grazing cows is essential, given that under adverse conditions cows can urdergo thermal stress (Kadzere et al., 2002). Besides economic impacts (St-Pierre et al., 2003), adopting mechanisms that mitigate environmental stressors in animal production may also address ethical concerns of consumers (Cardoso et al., 2018).

The benefits that silvopastoral systems (SPS) can bring to the environment (Barton et al., 2016; Castro and Fernández-Núñes, 2016;

* Correspondence to: Rua dos Funcionários, 1540 - Juvevê, Curitiba CEP 80035-050, Paraná, Brazil.
E-mail addresses: matheus-utfpr@hotmail.com (M. Deniz), karoltenffen10@hotmail.com (K.T. de Sousa), morosmi@hotmail.com (M.F. Moro), marcos.vale@ufpr.br (M.M. Vale), dittrich@ufpr.br (J.R. Dittrich), pinheiro.machado@ufsc.br (L.C.P. Machado Filho), maria.j.hotzel@ufsc.br (M.J. Hötzel).
[1] ORCID: 0000–0001-8079–0070

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44 SUPLEMENT E - PATENT: COMPUTER PROGRAM. REGISTRATION IN THE NATIONAL INSTITUTE OF INDUSTRIAL PROPERTY, BRAZIL.

REPÚBLICA FEDERATIVA DO BRASIL
MINISTÉRIO DA ECONOMIA
INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL
DIRETORIA DE PATENTES, PROGRAMAS DE COMPUTADOR E TOPOGRAFIAS DE CIRCUITOS INTEGRADOS

Certificado de Registro de Programa de Computador

Processo Nº: **BR512021000421-3**

O Instituto Nacional da Propriedade Industrial expede o presente certificado de registro de programa de computador, válido por 50 anos a partir de 1° de janeiro subsequente à data de 25/10/2019, em conformidade com o §2°, art. 2° da Lei 9.609, de 19 de Fevereiro de 1998.

Título: CowSystem

Data de publicação: 25/10/2019

Data de criação: 25/10/2019

Titular(es): UNIVERSIDADE FEDERAL DO PARANA

Autor(es): MATHEUS DENIZ; KAROLINI TENFFEN DE SOUSA; ISABELLE CORDOVA GOMES; JOÃO RICARDO DITTRICH

Linguagem: C++

Campo de aplicação: AG-10

Tipo de programa: GI-01; GI-04; LG-02; SO-07

Algoritmo hash: SHA-512

Resumo digital hash:
F6051FA0F542043A20E2C7C3F77F16262B2200587F781167305680E666498E99C86E52A2F9BB4CE190816E75912
56FC9EFADB4A0B66F4593EF931C11581A2357

Expedido em: 16/03/2021

Aprovado por:
Carlos Alexandre Fernandes Silva
Chefe da DIPTO

45 SUPLEMENT F - PATENT SUBMITTED TO REGISTRATION IN THE NATIONAL INSTITUTE OF INDUSTRIAL PROPERTY, BRAZIL.

Instituto Nacional da
Propriedade Industrial
Ministério da Economia

Consulta à Base de Dados do INPI

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2657	**07/12/2021**	2.10	-	- Número de Protocolo '870210109668' em 26/11/2021 13:12 (WB)

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46 SUPLEMENT G - ABSTRACT PUBLISHED IN THE “V WORKSHOP INTERNACIONAL DE AMBIÊNCIA DE PRECISÃO”

PRECISION LIVESTOCK FARMING FOR EVALUATION THE THERMAL ENVIRONMENT OF DAIRY COWS RAISED ON PASTURE

M Deniz[1], MM do Vale[1*], KT de Sousa[1], JR Dittrich[1], IC Gomes[2], JA Fabro[3]

[1]Universidade Federal do Paraná (UFPR), Departamento de Zootecnia, Laboratório de Inovações Tecnológicas em Zootecnia (LITEZ), Curitiba, PR – Brasil; [2]UFPR, Engenheira da Computação, afiliada ao LITEZ, Curitiba, PR – Brasil; [3]Departamento Acadêmico de Informática da Universidade Tecnológica Federal do Paraná, Curitiba, PR – Brasil

Resumo

O objetivo deste trabalho foi avaliar a eficiência de um protótipo de baixo custo em coletar dados microclimáticos acoplado ao corpo de vacas leiteiras criadas a pasto. A distribuição das vacas na pastagem acarretou variação na temperatura do ar, umidade relativa e temperatura de globo negro. Fato este que resultou em variação na temperatura superficial da pele entre as vacas, sendo que a vaca 2 de pelagem escura, obteve maior temperatura superficial da pele do que a vaca 1 de pelagem clara. O protótipo foi eficiente em coletar dados microclimáticos, sendo possível realizar coletas de dados individuais em bovinos a pasto.

Abstract

The objective of this work was to evaluate the efficiency of a low-cost prototype fixed on the back dairy cows raised on pasture in evaluating microclimatic variables. Cows' distribution at the pasture promoted variation in air temperature, relative humidity, and black globe temperature. This fact resulted in variation in body surface temperature. Cow 2 with a dark coat obtained higher body surface temperature than cow 1 with a light coat. The prototype was efficient in evaluating microclimatic variables, being possible to measure individual data the cattle on pasture.

Introduction

Inadequate interaction of environmental factors such as air temperature, relative humidity and solar radiation can promote heat stress. This condition is not desired in livestock because it can reduce animal production, besides causing an increase in rectal temperature, decline in feed intake, increased water intake, weight loss and even death in extreme cases.

In order to evaluate the real thermal comfort condition of animals, it is important to collect environmental variables as close as possible to animals [1]. This requires technologies that assist in the management of data collection.

The use of technologies in livestock has changed the way of operating and organizing the farms. Real-time data collection directly in the individual has been helping in decision making within production systems. Thus, the aim of this study was to evaluate the efficiency of a low-cost prototype in evaluating microclimatic variables of dairy cows raised on pasture.

Materials and methods

This study was approved by the Animal Use Ethics Committee of the Federal University of Paraná under protocol 083/2019.

The experiment was carried out in July 2019 at the Centro Paranaense de Referência em Agroecologia (CPRA), in Parana Stat - Southern Brazil (25°26'41"S, 49°11'33"W). For this experiment, the animals were kept in an open pasture paddock (1000 m^2) and had *ad libitum* access to the mineral salt and water trough.

The collection of variables at the animal’s level was performed by an autonomous prototype. This prototype is composed of three sensors coupled to a microcontroller and allocated in a watertight box (380 cm^3). The prototype was fixed on the back two crossbred dairy cows (Jersey / Holstein), 36 months old

47 SUPPLEMENT H - ABSTRACT PUBLISHED IN THE "V WORKSHOP INTERNACIONAL DE AMBIÊNCIA DE PRECISÃO"

KALMAN FILTER OPTIMIZES MPU-6050 SENSOR DIGITAL SIGNAL PROCESSING TO ANIMAL BEHAVIOUR MONITORING

M Deniz[1*], MM do Vale[1], KT de Sousa[1], IC Gomes[2], MF Ferraz[3], GS Berger[3], MA Wehrmeister[3]

[1]Universidade Federal do Paraná (UFPR), Departamento de Zootecnia, Laboratório de Inovações Tecnológicas em Zootecnia (LITEZ), Curitiba, PR – Brasil; [2]UFPR, Engenheira da Computação afiliada ao LITEZ, Curitiba, PR – Brasil; [3]Universidade Tecnológica Federal do Paraná, Laboratório de Engenharia de Sistemas Computacionais (LESC), Curitiba, PR – Brasil

Resumo
Automatizar avaliações de comportamento animal auxilia no desenvolvimento de sistemas de pecuária de precisão. Contudo, os sinais coletados podem sofrer ruídos que afetam a sua análise. O filtro de Kalman foi avaliado como ferramenta para aprimorar o processamento digital de sinais provenientes do sensor MPU-6050. O filtro de Kalman eliminou ruídos dos sinais coletados. Assim, houve melhora na eficiência do algoritmo que adquiriu e processou os dados do sensor MPU-6050. Tal melhora foi obtida através da mitigação do efeito dos ruídos captados pelo sensor propiciando sinais mais adequados para a análise no estudo do comportamento animal.

Abstract
The automation of animal behavior assessments assists with the development of precision livestock farming systems. However, the gathered signals can sometimes present noise thus affecting their analysis. The Kalman filter has been evaluated as a tool to improve digital signal processing from the MPU-6050 sensor. The Kalman filter removed the noise in the collected signals, thus increasing the efficiency of the algorithm that acquired and processed the MPU-6050 sensor data. Such an improvement is related to the mitigation of the effect of the sensor noise thus providing more suitable signals to be analyzed in the study of animal behavior.

Introduction

Evaluating farm animal activity has become a matter of great interest because the behavior indicates health and welfare status [1]. However, individual observation has limitations, as it demands great effort to identify individuals and understand their states. Therefore, automated systems to monitor the behavior are required [2].

To deal with such issues, the use of sensors in livestock research has rapidly increased in recent years [3]. The sensors allow monitoring many variables, especially the animal behavior. Using the accelerometer sensor is a good monitoring tool e.g. for estimating grazing [4], to predict calving [5], to detect lameness [6], and others.

Although the accelerometer sensor is widely used in precision livestock farming, this sensor is highly sensitive to vibrations and can produce uncertain results due to the noises. This situation is highly unwanted for decision making process. Thus, this study aims to evaluate the Kalman filter as a tool to improve digital signal processing from the Inertial Measurement Unit (MPU-6050) sensor.

Materials and Methods

This study was carried out at the "Laboratório de Inovações Tecnológicas em Zootecnia" (LITEZ) at the Federal University of Paraná, Curitiba. In this study, two commercial components were used: an integrated circuit MPU-6050 and an Arduino nano microcontroller. The

48 SUPPLEMENT I - ABSTRACT PUBLISHED IN THE “1 SEMANA DA PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ”

Archives of Veterinary Science ISSN 1517-784X
v.25, n.5 Esp. I Semana Acadêmica da Pós-Graduação em Ciências Veterinárias - UFPR (I SAPGCV), p. 114, 2020 www.ser.ufpr.br/veterinary

EM VACAS LEITEIRAS A IDADE É MAIS IMPORTANTE QUE O CHIFRE PARA DETERMINAR O ACESSO A SUPLEMENTAÇÃO NO COCHO

(For dairy cows age is more important than horns to determine the access to feed supplementation)

Matheus Deniz, Karolini Tenffen de Sousa, Deise Taborda Martins, Vitória Alves Branco Riezemberg, Barbara Haline Buss Baiak, Marcos Martinez do Vale, João Ricardo Dittrich

Universidade Federal do Paraná, Curitiba, Paraná, Brasil.

*Correspondência: matheus-utfpr@hotmail.com

RESUMO: A descorna é uma prática comum na bovinocultura de leite, na qual geralmente não são utilizadas técnicas, como anestesia e analgesia para aliviar a dor dos animais. No Brasil, a descorna é proibida na produção animal orgânica. Porém, existe preocupação com a presença de animais com chifres no rebanho, pois pode aumentar o risco de lesões durante a alimentação, devido as disputas por acesso ao alimento. No entanto, se adaptarmos as instalações e melhorar as condições de manejo, pode não ser necessário sujeitar os animais a esse procedimento doloroso. Assim, o objetivo deste estudo foi avaliar a influência da idade e a presença de chifre no acesso a suplementação no cocho. O estudo foi realizado na Estação de Pesquisa Agroecológica – CPRA, do Instituto de Desenvolvimento Rural do Paraná, em Pinhais, Brasil. Participaram deste estudo 27 vacas (7 com chifre e 20 mochas) mestiças (Jersolando) em lactação, com idade média de 54 ± 24 (média ± DP) meses e peso médio de 410 kg ± 41. As observações foram realizadas na área de alimentação por 13 dias não consecutivos em julho de 2019, com duração de uma hora (8h – 9h). Silagem de milho foi fornecida em um cocho de concreto (27 m) com um espaço linear de 1m/animal e água foi fornecida *ad libitum*. O tempo no cocho foi registrado por *scan-sampling* a cada um minuto e as interações agonísticas foram registradas sempre que ocorreram. Para análise dos dados, dividimos os animais em três categorias de acordo com a idade: jovens: 2-3 anos (n=8), intermediário: 4-5 anos (n=12) e mais velhos: 6-10 anos (n=7). Para eliminar a discrepância numérica entre as categorias de idade, a frequência no cocho (%) foi balanceada de acordo com o número de animais em cada categoria. Análises de influência foram realizadas por Modelos Lineares Generalizados e uma correlação de Spearman foi usada para examinar a relação entre as categorias e a frequência no cocho. Todas as análises foram realizadas com 95% de confiança pelo software R. Não houve diferença ($p>0,05$) entre o peso das três categorias; animais jovens apresentaram peso médio de 406,7 ± 45,4 kg, os intermediários pesaram 409 ± 42,1 kg e os mais velhos 404 ± 49,3 kg. Houve correlação ($p<0,05$) do tempo no cocho com a idade ($r = 0,37$) e presença de chifre ($r = -0,57$). Todos os animais com chifre se concentraram na categoria dos mais jovens e tiveram menor frequência no cocho ($p<0,05$). Os animais mais jovens foram as principais vítimas (70%) das interações agonísticas, seguido dos intermediários (44%) e os mais velhos (33%). Não houve diferença ($p>0,05$) na frequência no cocho entre os animais intermediários (80%) e os mais velhos (77%), porém os animais mais velhos foram os principais instigadores (67%), direcionando principalmente energia para deslocar os animais mais jovens para fora do cocho. A idade foi mais importante que a presença de chifre para determinar o acesso das vacas leiteiras ao suplemento no cocho.

Palavras-chave: bem-estar; cornos; etologia aplicada; hierarquia-social.

Agradecimentos: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela bolsa concedida ao primeiro autor. Ao Evandro M. Richter e João A. G. Hill pela oportunidade de realizar este trabalho no CPRA.

Nota: Este trabalho foi aprovado pela Comissão de Ética no Uso de Animais da Universidade Federal do Paraná sob protocolo número 083/2018.

49 SUPLEMENT J - ABSTRACT PUBLISHED IN THE 56TH ANNUAL MEETING OF THE BRAZILIAN SOCIETY OF ANIMAL SCIENCE

56ª Reunião da Sociedade Brasileira de Zootecnia

16 a 20 de Agosto de 2021

VIRTUAL

Silvopastoral system as an alternative to mitigate the decrease of milk production during hot seasons

Matheus Deniz*[1,2], Karolini T. de Sousa[1,2], Isabelle C. Gomes[2], Gabriel L. Z. Matthes[2], Thalessa N. Pereira [2], João R. Dittrich[1,2]
[1]Programa de Pós-Graduação em Zootecnia, Universidade Federal do Paraná (UFPR), Curitiba/PR – Brasil;
[2]Laboratório de Inovações Tecnológicas em Zootecnia, UFPR.
*Doctoral student – matheus-utfpr@hotmail.com

The thermal environment is directly related to the animals' life quality and the productivity of dairy farms. The aim of this study was to evaluate the thermal comfort index in two pasture systems during hot seasons (spring and summer) and thereby estimate economic losses in relation to the decrease of milk production. Two pasture systems evaluated were: treeless pasture (TLP) and silvopastoral system (SPS) with trees along the border fences. The experiment was carried out between September of 2020 and February of 2021 on a research center located in Pinhais, Paraná State, Brazil. During four non-consecutive days per month, microclimate variables of air temperature (AT, °C) and relative humidity (RH, %), were measured using four digital thermo-hygrometers with dataloggers. Two equipment were located at full sun exposure (center of the paddock), in the TLP. While in the SPS, one equipment was located at full sun exposure (distant from the trees), and the other was located at the shaded condition (1.5 m from the trees). Data collections were performed at a height of 1.3 m from the ground (height of the mass center of a Jersey adult cow), every 5 min. in each system (TLP and SPS). With the microclimatic data, we determined the Temperature and Humidity Index (THI). We used 20 L cow day^{-1} as reference value of milk production and THI for estimated the decrease of milk production (DMP), using the equation DMP = -1,075 - 1,736 * 20 L + 0,02474 * 20 L * THI. The economic losses were determined considering milk production, DMP, price of the milk, and days of the season (90 d). The model of economic losses was performed by Excel software, while the confirmatory analyses were performed using a generalized linear model by the statistical software R. The SPS presented the lowest mean values ($p<0.05$) of the microclimatic variables. In the spring, the difference between the systems was 3.1 °C for AT and 4.5% for RH, while in summer it was 3.7 °C and 6.1%. There was a difference ($p<0.05$) in the mean THI values between the systems (spring: SPS 69, TLP 74; summer: SPS 74, TLP 82). Both systems promoted a potential for thermal discomfort to animals in the summer and consequently triggered productive losses. The estimated of DMP in the TLP was 2.2 L cow day^{-1} in the spring, and 5 L cow day^{-1} in the summer. While in the SPS the estimated of DMP was 0.4 L cow day^{-1} in the spring and 1.8 L cow day^{-1} in the summer. The economic losses in the TLP was R$378 cow^{-1} during the spring, and R$859.50 cow^{-1} during summer. Meanwhile, in the SPS the economic losses were R$68.40 cow^{-1} during spring, and R$162 cow^{-1} during the summer. Thus, farmers should be aware of the environmental conditions in which the animals are raised and seek alternatives to mitigate the effect of the thermal environment. The SPS provided better environmental conditions for dairy cows, with the potential to minimize the economic losses of livestock by attenuating the decrease of milk production in hot climate regions.

Keywords: dairy cows, economic losses, THI, trees, shaded pastures.

50 SUPLEMENT K - ABSTRACT PUBLISHED IN THE INTERNATIONAL CONGRESS OF BIOMETEOROLOGY

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123 Views

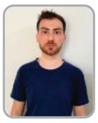
Back

Animal - Latin America

Influence of thermal environment on dairy cows' location in a silvopastoral system of a subtropical climate

! Wednesday, September 22, 2021 " 4:30 PM – 5:30 PM US EDT

Poster Presenter(s)



Matheus Deniz, PhD
student
Universidade Federal do Paraná
Curitiba, Parana, Brazil

Co-Author(s)

Karoliini de Sousa, PhD
student
Universidade Federal do Paraná
Curitiba, Parana, Brazil

Matheus Fernando Moro, Researcher
Researcher
Federal University of Santa Catarina
Florianópolis, Santa Catarina, Brazil

Marcos Martinez Vale, PhD
Professor
Universidade Federal do Paraná
Curitiba, Parana, Brazil

Joao Ricardo Dittrich, PhD
Professor
Universidade Federal do Paraná, Parana, Brazil

Maria J. Hötzel, PhD
Professor
Universidade Federal de Santa Catarina
Florianopolis, Brazil

Due to climate change heat abatement is a hot topic for pasture-based dairy systems. Silvopastoral systems promote a better thermal environment than treeless pasture for dairy cows. However, cows' use of shade can be influenced by the environmental thermal challenge. The aim of this study was to evaluate the relationship between black globe-humidity index (BGHI) and cows' location (shade or sun) in a silvopastoral system. The study was carried out between March 2020 and February 2021 during four non-consecutive cloudless days per month. In a silvopastoral system, we determined shaded (10.7m^2/cow) and sunny (98.4m^2/cow) areas. In each area, air temperature (°C), relative humidity (%), black globe temperature (°C), soil surface temperature (SST, °C), and 39 Jersey cows' location and posture (stand and lie down) were registered with 10 min. interval. BGHI was determined from the microclimate variables and grouped into classes (≤74 = thermal comfort; 75 – 78 = warning; and 79 – 84 = danger). BGHI, SST, and cows' location were analyzed using a mixed model with days and hours as random effects. In order to determine whether cows' location had a relationship with the BGHI index, a binomial regression model with logit link function was determined. The outcome of interest was the cows' location at the shaded or sunny areas (binary-dependent variable) associated with two independent variables (BGHI, and animals' posture). For interpretation purposes, the parameters are presented in odds ratio (OR) and $p<0.05$ was considered significant. Cows spent more time ($p < 0.05$) in

51 SUPLEMENT L – ABSTRACT PUBLISHED IN THE 2ND INTERNATIONAL ELECTRONIC CONFERENCE ON ANIMALS – GLOBAL SUSTAINABILITY AND ANIMALS: WELFARE, POLICIES AND TECHNOLOGIES

biology and life sciences forum

MDPI

Abstract

Silvopastoral systems as a sustainable alternative to mitigate the effects of climate change on farm level†

Matheus Deniz [1]*, Karolini T. de Sousa[1] Marcos M. do Vale[1], João R. Dittrich[1]; João A. G. Hill[2], Maria J. Hötzel[3]

[1] Laboratório de Inovações Tecnológicas em Zootecnia (LITEZ), Universidade Federal do Paraná, Rua dos Funcionários, 1540 - Juvevê, Curitiba – PR, 80035-050, Brazil; karoltenffen10@hotmail.com (K.T.S.); marcos.vale@ufpr.br (M.M.V.); dittrich@ufpr.br (J.R.D.)
[2] Estação de Pesquisa Agroecológica CPRA, Instituto de Desenvolvimento Rural do Paraná, Estr. da Graciosa, 6960 - Jardim das Nascentes, Pinhais - PR, 83327-055, Brazil; joaohill@idr.pr.gov.br (J.A.G.H.)
[3] Laboratório de Etologia Aplicada e Bem-Estar Animal (LETA), Universidade Federal de Santa Catarina, Rod. Admar Gonzaga, 1346 - Itacorubi, Florianópolis - SC, 88034-000, Brazil; maria.j.hotzel@ufsc.br (M.J.H.)
* Correspondence: matheus-utfpr@hotmail;
† Presented at the 2nd International Electronic Conference on Animals - Global Sustainability and Animals: Welfare, Policies and Technologies, 29 Nov – 23 Dec 2021

Abstract: Climate changes cause an increase in the duration and intensity of heatwaves and promotes a decrease in the time that cattle remain in thermal comfort zones. Silvopastoral systems can be considered a nature-based solution to mitigate the effects of climate change. The aim of this study was to estimate the thermal comfort of bovines during hot seasons (spring and summer) in a silvopastoral system compared to treeless pasture. The experiment was carried out between September of 2020 and February of 2021 in southern Brazil. Two pasture systems were evaluated (4 non-consecutive days per month): treeless pasture (TLP) and silvopastoral system (SPS) with trees along the border fences. Two sets of autonomous sensors were located in each system (TLP - center of the paddock and SPS - full sun and 2 m away from the trees), to measured microclimate variables used to calculate the bioclimatic indicators of black globe-humidity index (BGHI), radiant thermal load (RTL), and heat load index (HLI). All data were analyzed using a mixed model with days and hours as random effects using the statistical software R. There was an influence of the system ($p<0.001$) on the bioclimatic indicators. On average the SPS was ~80% ($p<0.001$) more likely to present lower values of bioclimatic indicators than the TLP. The average values of all bioclimatic indicators differed ($p<0.001$) between the systems; TLP: BGHI = ~78; RTL = ~581, and HLI = ~59; SPS: BGHI = ~72; RTL = ~439, and HLI = ~47. In TLP all bioclimatic indicators were above the threshold for heat stress for bovines, promoting a challenge thermal environment for pasture-based production. In conclusion, the SPS provided a better thermal environment for pasture-based systems when compared to TLP, indicating that it can mitigate the effects of heat during the spring and summer of subtropical climate.

Keywords: Heat abatement; Thermal comfort; Wood pasture

Citation: Deniz, M.; de Sousa, K.T.; Vale, M.M.; Dittrich, J.R. Hill, J.A.G.; Hötzel, M.J. Silvopastoral systems as a sustainable alternative to mitigate the effects of climate change on farm level. *Biol. Life Sci. Forum* **2021**, *1*, x. https://doi.org/10.3390/xxxxx

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52 SUPLEMENT M – APPROVAL PROTOCOL OF THE ANIMAL ETHICS COMMITTEE OF THE UNIVERSIDADE FEDERAL DO PARANÁ

UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o protocolo número 083/2018, referente ao projeto **"Respostas fisiológicas e comportamentais de vacas leiteiras em sistema agroecológico de criação",** sob a responsabilidade **João Ricardo Dittrich** – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de Outubro, de 2008, do Decreto nº 6.899, de 15 de julho de 2009,e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), efoi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DO SETOR DE CIÊNCIAS AGRÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ - BRASIL, com grau 1 de invasividade, em reunião de 07/11/2018.

Vigência do projeto	Maio/2019 até Janeiro/2021
Espécie/Linhagem	*Bos taurus taurus* (bovino)/Jersey
Número de animais	50
Peso/Idade	180 – 400 kg/1,5 – 4 anos
Sexo	Fêmea
Origem	Centro Paranaense de Referência em Agroecologia, Pinhais, Paraná, Brasil.

CERTIFICATE

We certify that the protocol number 083/2018, regarding the project **"Physiological and behavioral responses of dairy cows in an agroecological system"** under **João Ricardo Dittrich** supervision – which includes the production, maintenance and/or utilization of animals from Chordata phylum, Vertebrata subphylum (except Humans), for scientific or teaching purposes – is in accordance with the precepts of Law nº 11.794, of8 October, 2008, of Decree nº 6.899, of 15 July, 2009, and with the edited rules from Conselho Nacional de Controle da Experimentação Animal (CONCEA), and it was approved by theANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES CAMPUS OF THE UNIVERSIDADE FEDERAL DO PARANÁ (Federal University of the State of Paraná, Brazil), with degree 1 of invasiveness, in session of07/11/2018.

Duration of the project	May/2019 until January/2021
Specie/Line	*Bos taurus taurus* (bovine)/Jersey
Number of animals	50
Wheight/Age	180 – 400 kg/1.5 – 4 years
Sex	Female
Origin	Centro Paranaense de Referência em Agroecologia, Pinhais, Paraná, Brazil.

Curitiba, 07 de novembro de 2018

Chayane da Rocha

Chayane da Rocha

Coordenadora CEUA-SCA

53 SUPLEMENT N - PROROGATION OF APPROVAL PROTOCOL OF THE ANIMAL ETHICS COMMITTEE OF THE UNIVERSIDADE FEDERAL DO PARANÁ

UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

OFÍCIO Nº 050/2020

Para: João Ricardo Dittrich
Assunto: Protocolo 083/2018

Prezado(a) pesquisador(a),

Após avaliação sobre seu pedido de prorrogação do projeto intitulado "**Respostas fisiológicas e comportamentais de vacas leiteiras em sistema agroecológico de criação"** de janeiro de 2020 para janeiro de 2022, esta Comissão informa que tal solicitação foi aprovada.

Simone Tostes de Oliveira Stedile
Coordenadora da Comissão de Ética
no Uso de Animais
SCA - UFPR