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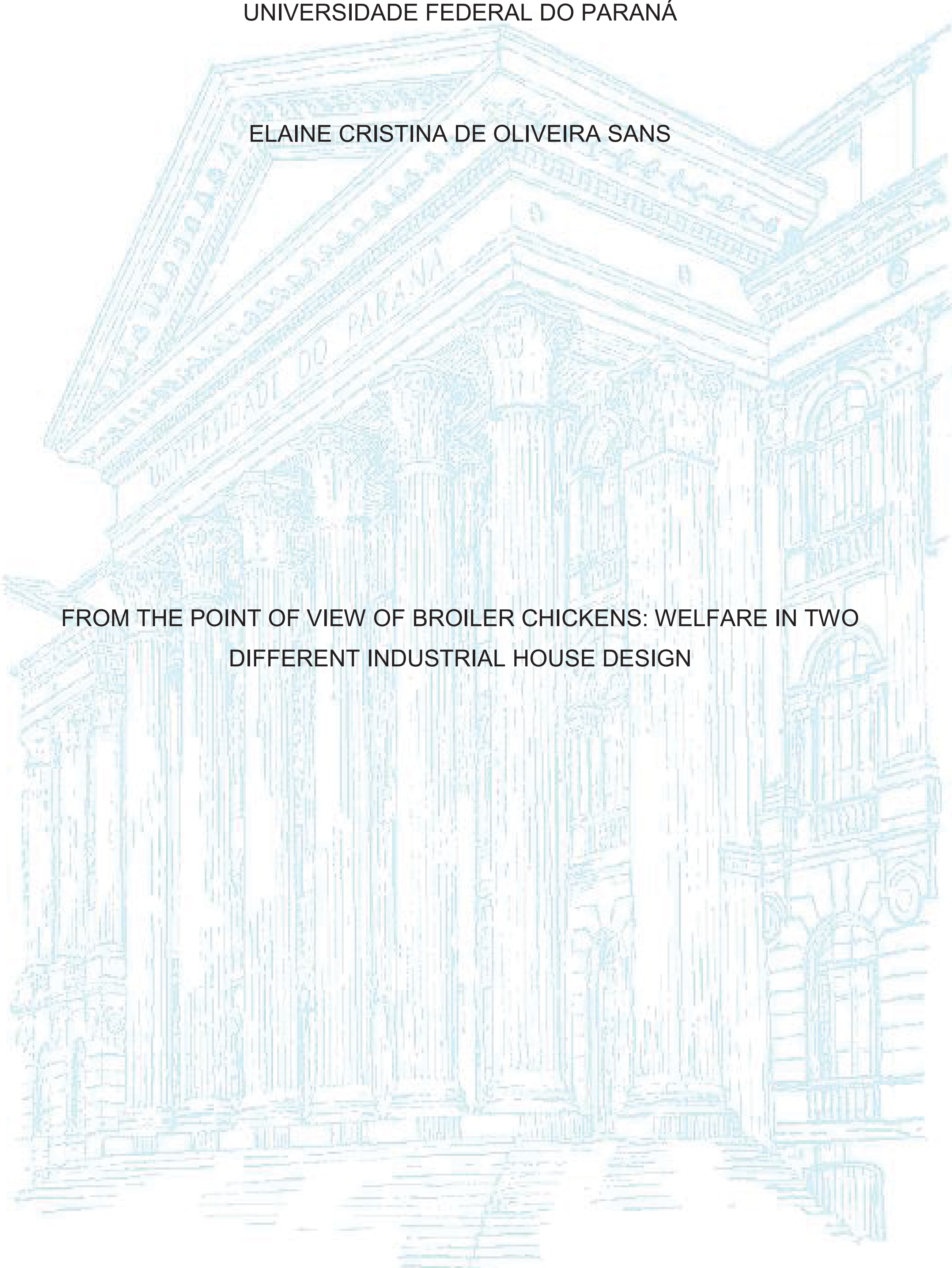

ELAINE CRISTINA DE OLIVEIRA SANS

FROM THE POINT OF VIEW OF BROILER CHICKENS: WELFARE IN TWO DIFFERENT INDUSTRIAL HOUSE DESIGN

CURITIBA

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ELAINE CRISTINA DE OLIVEIRA SANS

FROM THE POINT OF VIEW OF BROILER CHICKENS: WELFARE IN TWO DIFFERENT INDUSTRIAL HOUSE DESIGN

Tese apresentada ao curso de Pós-graduação em Ciências Veterinárias, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutora em Ciências Veterinárias.

Orientadores: Prof.ª Dr.ª Carla Forte M. Molento
Prof. Dr. Frank Andre M. Tuyttens

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIAS VETERINÁRIAS da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **ELAINE CRISTINA DE OLIVEIRA SANS** intitulada: **From the point of view of broiler chickens: welfare in two different industrial house designs.**, sob orientação da Profa. Dra. CARLA FORTE MAIOLINO MOLENTO, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

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“It is not what we think or say that matters, it is what we do.”

John Webster

RESUMO

A ciência do bem-estar animal está se tornando cada vez mais importante, pois contribui para o entendimento e atendimento das necessidades físicas e mentais dos animais. A indústria de carne envolve um grande número de frangos de corte, sendo o maior em relação a animais vertebrados terrestres. Assim, há um proporcional potencial de existir sofrimento nos sistemas intensivos e nos diferentes tipos de instalações utilizadas na criação de aves, tornando importante a identificação de pontos críticos de bem-estar que podem orientar as práticas de manejo. Desta forma, este trabalho objetivou avaliar o grau de bem-estar de frangos de cortes mantidos nos dois principais tipos de galpão utilizados na região Sul do Brasil (dark-house/CS *vs* semi-climatizado/OS), mantidos no clima subtropical, e como suas características influenciam sobre as condições ambientais internas como umidade relativa, temperatura, velocidade do ar, amônia, dióxido de carbono, iluminância e umidade da cama, e sobre os indicadores baseados nos animais como as dermatite de contato nas áreas do peito e abdômen, limpeza de penas, pododermatite, queimadura de jarrete, claudicação, fraturas, arranhões, aves mortas durante o transporte, doenças, repertório comportamental e estados afetivos. A tese está organizada em seis capítulos: 1) Apresentação; 2) Bem-estar de frangos de corte criados em dois diferentes tipos de galpão; 3) Bem-estar de frangos de corte criados em dois diferentes tipos de galpão durante o inverno no Sul do Brasil; 4) Heterogeneidade interna em relação ao bem-estar de frangos de corte criados em dois diferentes tipos de galpão e duas estações do ano, no clima subtropical do Sul do Brasil; 5) Do ponto de vista das aves: que diferença faz uma janela?; 6) Considerações finais. Durante as estações do verão/outono, os galpões do tipo OS apresentaram menores restrições de bem-estar em relação aos galpões CS, mas ambas as instalações evidenciaram importantes problemas de bem-estar das aves, em relação às más condições ambientais, restrições comportamentais e injúrias. No estudo realizado durante o inverno, os galpões CS ofereceram restrições menos severas de bem-estar das aves, especialmente nos indicadores de saúde, enquanto nos galpões OS as restrições foram menores em relação aos indicadores comportamentais e estados emocionais positivos. No quarto capítulo, por meio da geoestatística, foram identificadas áreas com maiores problemas de bem-estar animal, sendo nos galpões CS na região Oeste, próximo aos exaustores, e em galpões OS, na direção da ventilação positiva efetuada pelos ventiladores. O teste de preferência indicou que as aves preferem uma área com disponibilidade de luz natural fornecida por meio de janelas, combinada a luz artificial e que no ambiente com luz natural, seu repertório comportamental também foi diferente. A presente tese permitiu identificar pontos críticos de bem-estar de frangos de corte, de acordo com os tipos de galpão mais comumente utilizados no Sul do Brasil, quais as áreas específicas dentro de cada tipo de galpão precisam de mais atenção e que, uma das principais características que diferencia os dois tipos de instalações, a iluminância, interfere na escolha, no comportamento e no bem-estar das aves. Os resultados apresentados podem auxiliar na tomada de decisão em relação ao planejamento de ações que favoreçam o aumento do grau de bem-estar dos frangos, pois reiteram os problemas enfrentados pelos frangos criados em sistemas intensivos, independentemente de tipo de galpão. Este cenário crítico de bem-estar animal exige considerações adicionais sobre ética animal em relação à produção de alimentos.

Palavras-chave: ambiente, comportamento, luz natural, galpão, sistema industrial, teste de preferência.

ABSTRACT

The science of animal welfare is becoming increasingly important, as it contributes to the understanding and meeting the physical and mental needs of the animals. The poultry meat industry involves a large number of broiler chicken individual animals, the largest one for terrestrial vertebrates used for food production. Thus, there is a proportional potential for suffering in poultry intensive housing systems, and this it becomes important identifying critical welfare points which may guide best management practices. Thus, this thesis aimed to assess broiler chickens' welfare that were reared in two main house designs used in Southern of Brazil (closed-sided houses/CS *vs* open-sided houses/OS), a subtropical climate, and how their features may influence on internal environmental indicators such as relative humidity, temperature, air velocity, ammonia and carbon dioxide concentrations, illuminance and litter moisture, and on animal-based indicators such as contact dermatitis on the breast and abdominal areas, bird soiling, footpad dermatitis, hock burn, lameness, fractures, bruising, scratches, death on arrival, diseases, behaviour and affective states. The thesis is organized in six chapters: 1) Presentation; 2) Welfare of broiler chickens reared under two different types of housing; 3) Welfare of broiler chickens reared in two different use types during the winter season in Southern Brazil; 4) In-barn heterogeneity of broiler chicken welfare in two industrial house designs and two seasons in Southern Brazilian subtropical climate; 5) From the point of view of the chickens: what difference does a window make?; 6) Final considerations. During summer/autumn, fewer animal welfare restrictions were observed in OS as compared to CS houses; however, both presented important welfare problems, evidenced by poor environmental conditions, behavioral restrictions and injuries. During winter, CS houses seemed to offer fewer welfare problems in terms of the health indicators; however, OS houses showed fewer behavioral restrictions and higher positive emotional states. In the fourth chapter, by geostatistics, it was observed that worse welfare problems was in the West direction, which in CS means near exhaust fans and in OS houses the direction of positive-pressure mechanical ventilation by fans. The preference test indicated that birds preferred an area with natural light, provided through the availability of windows, combined with artificial light, and in the area with natural light, their behavioral repertoire was different. This thesis contributes to identify critical points of broiler chickens' welfare, according to the house types most commonly used in Southern Brazil, which specific areas within each house types need care and one of the main characteristics that differentiates the poultry houses, illuminance, interfere in the birds' preference, their behavior and welfare. These results may assist in decision-making in relation to the planning of actions that may improve the welfare of broiler chickens, as these results reiterate the problems faced by chickens raised in intensive systems, regardless of the house design. This critical animal welfare scenario calls for additional animal ethical considerations concerning food production.

Keywords: behaviour, environment, industrial system, natural light, poultry house, preference test.

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1. PRESENTATION

The science of animal welfare is increasingly important, as society demands for improvements in the life quality of sentient animals under human care. Accordingly, the professionals involved in the animal production industry have the responsibility to alleviate animal suffering (WEBSTER, 1998; WEBSTER, 2016).

The poultry meat industry entails an extremely large number of animals, in a scale of billions per year. The broiler chicken industry involves the largest number of individual animals considering terrestrial vertebrate animals that are used for food production. Current scientific evidence clearly demonstrates that vertebrate animals are sentient beings, capable of experiencing different feelings which are relevant for them. This fact puts high priority for chicken production systems in terms of immediate demands for animal welfare improvements (BROOM, 2001; ROWE; DAWKINS; GEBHARDT-HENRICH, 2019).

Poultry production is economically relevant to Brazil, distributed across all regions of the country. Although there is no clear data regarding the number of each type of barns used, Brazilian broiler chickens are raised in different barns, which may be categorized as conventional, semi- or fully-climatised houses. The conventional and semi-climatised barns, abbreviated as OS in this thesis, generally consist of open sided walls with curtains and both artificial and natural light, and may be equipped with fans (positive pressure) and sprinklers. The Brazilian climatised barn, abbreviated as CS, is featured by artificial lighting, negative pressure and evaporative cooling systems, exhaust fans and sprinklers, and fully curtain-closed sides. Such curtains may be doubled and vary in colour, such as black, blue, green or yellow (BARACHO et al., 2018; LIMA; SILVA, 2019).

The development of this thesis was motivates by the changes that are occurring in Brazil, where the industry is increasingly adopting closed-sided houses. Although this type of barn seems to offer better environmental conditions, it comes with higher bird densities and much less illuminance. Thus, what are the positive and negative aspects of each barn type regarding animal welfare, and how can we identify and improve them? How do seasons, internal bird location and levels of illuminance influence the effects of each house type on bird welfare and choice opportunities? Based on real on-field welfare challenges, this thesis aimed at assessing the welfare of broiler chickens reared in two main house designs used in Southern of Brazil, closed- (CS) and the open- (OS) sided barns, and how their characteristics influence bird life quality, with some insights on the point of view of the birds themselves.

Positive and negative points may be observed in each house design, but some items are not yet fully clarified. For example, it is not known how the animal-based and environmental indicators of welfare behave according to the house type, seasonality and internal area of each barn. Another important point, moving to the opposite direction regarding animal welfare recommendations, refers to one of the main differences between the house designs, the illuminance (SOUZA et al., 2015), and how important this resource may be from the point of view of the chickens.

Chapter 2 presents the study regarding chicken welfare in the investigated house designs (CS and OS houses), during summer and autumn seasons. We observed animal welfare restrictions in three animal-based indicators in OS (air velocity, prevalence of scratches and behaviors classified as others) as compared to five indicators in CS houses (illuminance, NH_3 and CO_2 concentrations, contact dermatitis on the breast and abdominal areas, and exploratory behaviour). However, both houses presented important welfare problems, as evidenced by poor environmental conditions, considerable behavioral restrictions and a high prevalence of injuries. Results from this chapter were presented in II Congreso Latinoamericano de Comportamiento y Bienestar Animal – ISAE 2020 (APPENDIX II). This study was accepted for publication in the Animal Welfare Journal (APPENDIX III).

The same house designs and animal welfare indicators were also assessed during the winter season as presented in Chapter 3. This time, we observed that CS houses seemed to offer fewer welfare problems, especially in terms of health and environmental indicators (contact dermatitis on the breast and abdominal areas, bird soiling, hock burns, air velocity, NH_3 concentration). The OS houses showed better results in fewer behavioral restrictions and more positive emotional states (inactivity and drinking behaviours and QBA). This study is published in the British Poultry Science Journal (APPENDIX IV).

Chapter 4 aimed at identifying the in-barn distribution, using geostatistics, the assessment of a variety of animal welfare indicators, including the effects of season. According to our results, a systematic spatial distribution of increased welfare problems was identified as heading from the middle of the house towards the West end in both house types. This outcomes were observed for three environmental (temperature, NH_3 and CO_2 concentrations) and three health welfare indicators (hock burn, bird soiling, and footpad dermatitis). This study is published in the Livestock Science Journal (APPENDIX V).

Chapter 5 refers to the study investigating bird preference regarding illuminance, one of the main differences found between the house designs assessed in this thesis. The results of this preference test indicated that birds prefer an area with both natural and artificial light together, compared to an area with only artificial lighting. This preference alters depending on bird age and seems influenced by the heating light. In the area with both natural and artificial lighting, bird behavioral repertoire was richer, confirming that the presence of natural light is important for bird welfare. In addition, birds also used the darker area, which means that it is important, from their point of view. These results suggest that broiler chickens must have choices in terms of illuminance across the barn.

Final considerations, main conclusions and contributions regarding our results and broiler chicken welfare are presented Chapter 6.

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2. WELFARE OF BROILER CHICKENS REARED UNDER TWO DIFFERENT TYPES OF HOUSING

RESUMO

Comparamos o bem-estar de frangos de corte mantidos em sistemas intensivos e em dois tipos de galpão, dark-house (CS) *vs* semi-climatizado (OS), no Sul do Brasil. Foram avaliados 10 lotes de frangos de corte em cada tipo de instalação. As avaliações foram divididas em categorias: i) indicadores de saúde: dermatite de contato nas áreas do peito e abdômen, limpeza da ave, pododermatite, queimadura de jarrete, claudicação, fraturas, arranhões, aves mortas durante o transporte e doenças; ii) indicadores ambientais: umidade relativa, temperatura, velocidade do ar, concentrações de amônia (NH_3) e dióxido de carbono (CO_2), intensidade de luz e umidade da cama; iii) indicadores comportamentais: comportamento da ave e teste do toque; iv) estados afetivos: avaliação qualitativa do comportamento. Os galpões CS em relação aos OS, apresentaram piores resultados para dermatite de contato nas áreas do peito e abdômen, menor prevalência do comportamento exploratório, maiores concentrações de NH_3 (11,2[±6,8] *vs* 7,5[±3,9] ppm) e CO_2 (1124,9[±561,5] *vs* 841,0[±158,0] ppm), baixa intensidade luminosa (6,9[±6,3] *vs.* 274,2[±241,9] lx); enquanto os galpões OS apresentaram alta prevalência para arranhões e comportamento de ofego e menor velocidade do ar (2,1[±0,7] *vs* 1,1[±1,0] m s^{-1}). A densidade (13.9[±0.4] CS e 12.0[±0.3] aves/m^2 OS) provavelmente influenciou alguns resultados. Apesar dos galpões OS apresentarem menores restrições no bem-estar dos frangos de corte, de acordo com os cinco indicadores comparados aos três observados nos galpões CS, ambas as instalações apresentaram importantes problemas de bem-estar, evidenciados pelas más condições ambientais, restrições comportamentais e injúrias.

Palavras-chave: avicultura, comportamento, dark-house, frigorífico, semi-climatizado. verão/outono.

ABSTRACT

We compared closed- and open-sided industrial houses with respected to the welfare of broiler chickens in Southern Brazil. Ten flocks from each design were evaluated and measures divided into the following categories: i) bird health: contact dermatitis on the breast and abdominal areas, bird soiling, foot-pad dermatitis, hock burn, lameness, fractures, bruising, scratches, dead on arrival, and diseases; ii) environmental indicators: relative humidity, temperature, air velocity, ammonia (NH_3) and carbon dioxide (CO_2) concentrations, light intensity, and litter moisture; iii) behaviour: bird behaviour, and touch test; iv) affective states: qualitative behaviour assessment. Closed-sided houses showed worse contact dermatitis on the breast and abdominal areas, lower exploratory behaviour prevalence, higher NH_3 (11.2[±6.8] *vs.* 7.5[±3.9] ppm) and CO_2 (1124.9[±561.5] *vs.* 841.0[±158.0] ppm), lower light intensity (6.9[±6.3] *vs.* 274.2[±241.9] lx), while open-sided houses had a higher prevalence for scratches and panting behaviour, and lower air velocity (2.1[±0.7] *vs.* 1.1[±1.0] m s^{-1}). Stocking density of (13.9[±0.4] and 12.0[±0.3] per m^2 for closed- and open-sided houses, respectively, likely influenced some results. Even though open-sided houses presented fewer animal welfare restrictions (according to five indicators as opposed to three for closed-sided houses) both revealed important welfare problems, evidenced by poor environmental indicators, behavioural restrictions and injuries.

Keywords: behaviour, dark-house, environment, poultry, semi-climatised, slaughter, summer/autumn.

2.1 INTRODUCTION

Poultry is the most traded livestock species in the world, in terms of numbers of animals involved and meat tonnage, and Brazil is one of the leading producers and exporters. In 2020, around 5.9 billion of birds were slaughtered (IBGE, 2021) in Brazil, and the country produced 13.8 million tons of poultry meat, behind only the US (with 20.2 million tons) and China (14.6 million tons; ABPA, 2021). Due to the numbers of animals involved, poultry production becomes a major priority regarding animal welfare initiatives (BROOM, 2001; ROWE; DAWKINS; GEBHARDT-HENRICH, 2019). Improvements may stem from consumer and market pressure, company interests, new policies, funding availability, country and regional specificities and climate as well as individual specifications on-farm details such as house design and management.

No standard system is in place for raising broiler chickens in developing countries. However, there are concerns as regards striking a balance amongst farm maintenance conditions, animal welfare and production sustainability (LIMA et al., 2020). The Brazilian poultry industry utilises multiple systems with different house sizes and partial or absolute control over indoor environmental conditions. Most Brazilian broiler chickens are reared in open-sided poultry houses, so-called conventional and semi-climatised houses, with fans and access to natural lighting, combined with adjustable polypropylene curtains (PARANHOS DA COSTA; LIMA; SANT'ANNA, 2017). Closed-sided houses are fully enclosed by fixed curtains or walls and thermal insulation panels (OLANREWAJU et al., 2010), and are usually equipped with negative pressure and evaporative cooling systems, exhaust fans and sprinklers, and exclusive artificial lighting (ABREU; ABREU, 2011; OLANREWAJU et al., 2010; BARACHO et al., 2018). There are concerns about the lighting: for example, 75% of animal welfare experts studied by Rioja-Lang et al. (2020) agreed on the potential negative impact of artificial lighting regimes on poultry welfare; there is also concern from consumers regarding lighting regime (VANHONACKER et al., 2009). A number of authors recognise the importance of light, especially natural lighting, and offer recommendations for the inclusion of windows in closed-sided poultry house designs (BAILIE; BALL; O'CONNELL, 2013; EU, 2017; BAILIE; IJICHI; O'CONNELL, 2018). However, in direct contrast to this, Souza et al. (2015a) described that out of 15 poultry export companies in the State of Paraná, Brazil, 14 had declared an intention to increase their numbers of closed-sided houses. Despite the increased use of negative pressure systems for broiler chicken production, Lima et al. (2020) recommended open-sided poultry houses, due to the benefits associated with natural ventilation and higher litter quality. On the other hand, Rovaris et al. (2014) observed better control of environmental indoor conditions as well as improvement bird performance when rearing occurred in closed-sided houses; however, there was also a higher prevalence of foot calluses, probably due to the high stocking density practiced in this type of housing. In general, open-sided houses may allow for increased animal behaviour possibilities due to the access to natural light; however, the birds may suffer from thermal stress (BAILIE; BALL; O'CONNELL, 2013; LIMA; SILVA, 2019).

Regardless of the poultry house design, it is important that animal welfare levels are acceptable. Issues such as leg problems and contact dermatitis are amongst the major problems faced by broiler chickens (EFSA, 2010) and these may be influenced by type housing. Additionally, in both designs, indoor conditions such as temperature, relative humidity, air velocity, litter quality, light intensity and gases affect animal welfare. There are acceptable ranges for indicators such as relative humidity (45-70%), light intensity (at least 20 lx), carbon dioxide concentration (<3,000 ppm) and ammonia concentration (10-20 ppm; EFSA, 2012a; RSPCA, 2017). High gas concentrations increase the susceptibility to respiratory diseases (NÄÄS et al., 2007) and poor litter quality may lead to foot-pad dermatitis (DE JONG; GUNNINK; VAN HARN, 2014). Curtain management in open-sided poultry houses is reported as important for better air quality (LIMA et al., 2020), and it may also influence lighting, which is considered crucial in regulating broiler chicken production and welfare (EFSA, 2012a). According to House et al. (2020), when birds were reared in environments illuminated with lighting emitting diode (LED) supplemented by ultraviolet light, they showed decreased stress susceptibility and fear responses, indicating improved welfare and suggesting lighting to be an important factor to consider when comparing types of housing. Furthermore, data on injuries such as scratches, bruises and fractures may assist the detection of on-farm critical points of animal welfare that may lead to broiler chicken suffering (ALLAIN et al., 2009). Injuries may be assessed at the slaughterhouse during carcase inspection, potentially contributing to the overall assessment of broiler chicken welfare (SOUZA et al., 2018a).

In addition to monitoring physical health, behavioural observations are important, as they may be an essential tool in helping to understand environmental effects on animal welfare (PEREIRA et al., 2005). The assessment of emotional states and human-animal relationships may also assist the improvement of management practices. As guiding principles, it seems fair to consider that chickens seek safety, comfort, absence of fear, pain and diseases, access to food, water and light, and the expression of positive behaviours such as dustbathing, scratching and foraging (BUTTERWORTH, 2018).

Considering a broader range of opinions, the increased attention to animal welfare by citizens, politicians and farmers appears linked to the increasing numbers of animal welfare definitions, which may relate to different values regarding animal welfare (LUNDMARK et al., 2014). For instance, according to Miele, Evans (2006), consumers place greater emphasis on natural living conditions, while scientists are more concerned with the absence of suffering. However, these differences do not prevent meaningful animal welfare assessment, based both on ethical and scientific information (LUNDMARK et al., 2014; LUNDMARK; BERG; RÖCKLINSBERG, 2018). In addition, irrespective of differing priorities, there is a recognition of the importance of assessing animal welfare using animal-based indicators (ANONYMOUS, 2012). Thus, much can be learned and improved by regular animal welfare assessment, even though it is not always possible to reach consensus when comparing situations in which different aspects of welfare have been compromised.

Thus, for a variety of reasons, then, animal welfare is a complex concept and its assessment relies on a variety of indicators. Additionally, many managerial actions, including house design, will have consequences of animals' welfare. It is important that those involved in the production chain consider birds' needs, not to mention specific regional characteristics before new housing designs from other countries are implemented, with climatic, economic and cultural conditions that differ greatly from those seen in Brazil (ABREU; ABREU, 2011; EU, 2015). To provide support for such decision-making, this is the first research comparing poultry houses from the perspective of bird welfare that sought specifically to assess the effect of closed- (CS) and open-sided (OS) poultry house designs on broiler chicken welfare in Southern Brazilian conditions.

2.2 MATERIAL AND METHODS

2.2.1 Bird husbandry

The farms were selected according to availability, taking into account bird age and CS and OS houses (only those CS houses with black curtains and exclusive use of artificial lighting were selected). From March to April 2019, a period incorporating the end of summer and the beginning of autumn, in the West of Santa Catarina State, South of Brazil, ten CS and ten OS poultry houses from the same company were visited to assess bird welfare. External temperatures ranged between 20.5 and 34.0°C, relative humidity between 38 and 99%, air velocity between 0.0 and 1.6 m s^{-1} and light intensity between 848 and 6,900 lx, as measured outside the barns during visits. A brief farmer questionnaire and flock records were used to obtain general information such as initial number of birds, number of birds at the visit, their age, breed, as well as mortality and culling rates. The same animal scientist, with experience in poultry welfare and trained since 2011 in the use of the Welfare Quality® protocol for poultry, performed all on-farm assessments.

The participant farms raised male Cobb MX (nine CS and six OS houses) and Ross TM4 (one CS and four OS houses) and operated in an integrated system within the same company. The birds were evaluated between 33 and 36 days of age, at a means of 6 (±2) days before slaughter. The summary description of the studied units per house design is shown in TABLE 1.

TABLE 1 - MAIN CHARACTERISTICS OF TEN CLOSED- AND TEN OPEN-SIDED POULTRY HOUSES INCLUDED IN THE STUDY AND ASSESSED FROM MARCH TO APRIL 2019, IN THE WEST OF SANTA CATARINA STATE, SOUTH OF BRAZIL.

Variable	Closed-sided houses (n=10)	Open-sided houses (n=10)	*P*
Stocking density, birds/m^2	13.9±0.4	12.0±0.3	<0.001
House size, m^2	1,631±409	1,200±300	0.001
Flock size, number of birds at visit	34,940±15,919	20,563±10,221	0.013
Age at visit, d	33.9±0.3	34.5±1.2	0.745
Age at slaughter, d	39.0±2.4	41.0±1.8	0.133
Body weight at slaughter, kg	2.74±0.14	2.79±0.10	0.189

Mortality (%)	2.1±1.3	2.9±0.8	0.515
Culls (%)	1.2±0.7	0.8±0.4	0.951
Re-used litter (number of flocks/litter)	7.2±3.5	4.0±2.7	0.016

All CS houses presented black curtains as fixed material to supplement partial walls and transform the buildings into CS houses; negative ventilation, exhaust fans, sprinklers, light intensity controllers, heating system with automatic control and, in the case of four CS houses, air inlets were also present. The OS houses were semi-climatised, showing laterals with wire mesh covered by double yellow (nine OS houses) or blue (one OS houses) roll-up curtains, positive ventilation by fans, sprinklers, natural and artificial lighting. The company recommended an intermittent lighting programme from the age of 22 days until pre-slaughter, for both CS and OS houses, exposing the birds to 16-18 h of artificial lighting in CS, and natural light complemented with artificial lighting in OS houses. All farms used LED, incandescent, fluorescent, or mixed light types in the same unit, wood-shaving litter and automatic (ten CS and nine OS houses) or manual feeders (one OS houses; FIGURE 1).

FIGURE 1 - VIEW OF A CLOSED-SIDED (a) AND OPEN-SIDED (b) POULTRY HOUSE IN THE WEST OF SANTA CATARINA STATE, SOUTH OF BRAZIL.

To optimize the data collection time, in 6/10 CS and 5/10 OS farms, which maintained more than one poultry house with comparable conditions, behavioural data were recorded in one house, while other animal welfare indicators were collected in another. On farms with only one house available, data collection started with the behavioural video-recording and, after recording ended, other animal welfare indicators were evaluated. As a result, a total of 31 houses were evaluated, comprising the collection of complete data from 20 farms.

2.2.2 Health assessment and environmental indicators

Welfare assessments were performed between 0930 and 1740h, and the mean duration for bird health assessment was 185 (±48) min per flock. The collected on-farm health indicators were contact dermatitis on the breast and abdominal areas, scored on an ordinal scale from 0 (absence) to 3 (severe), bird soiling from 0 to 3, foot-pad dermatitis from 0 to 4, and hock burn from 0 to 4, assessed on the same sample of 150 birds per flock by the same assessor (WELFARE QUALITY®, 2009; SOUZA et al., 2018b). Lameness was assessed in another sample of 150 birds, from 0 (normal gait) to 5 (unable to walk; WELFARE QUALITY®, 2009). The assessment was performed throughout the house, which was divided into 30 equidistant locations, with ten randomly selected birds per location, giving a total of 300 birds assessed per flock.

Health indicators were also collected at the slaughterhouse from four CS and five OS houses. All these flocks were slaughtered in the same slaughterhouse. Two assessors, both with previous experience in collecting animal welfare data at slaughterhouses, were responsible for this phase. For harmonisation of procedures, the assessors were trained in broiler chicken lesion classification with the same pictures showing fractures, bruising and scores of scratches. To accommodate assessment of the high-speed line, selected carcases were assessed, identified by the colour of the bird's hanging hook, which was randomly selected. This was possible because, in the studied slaughterhouse, hooks were often different colors, which meant an interspace between the same coloured hooks of on average, ten birds or 5 s. This skipping method allowed assessment to be carried out at a slower rhythm compared to the line speed (SOUZA et al., 2018b). Due to the speed of the slaughter line and the complexity of certain indicators, the observer assessed one indicator at a time. A total of 100 carcasses were assessed for the presence of fractures and a further 100 carcasses for the presence of bruises (adapted from LUDTKE et al., 2010). Scratches were scored from 0 (absence) up to score 3 (severe; SOUZA et al., 2018b) in 100 additional carcases, giving a total of 300 carcases assessed per flock.

Data provided by the slaughterhouse regarding dead on arrival (DOA), total and partial carcass condemnation for ascites, arthritis, dermatosis, myopathy and air sacculitis were analyzed. For two OS houses, it was not possible to assess data for arthritis, dermatosis and air sacculitis, as these data were not available.

Environmental parameters were collected to characterize the indoor living conditions in all units simultaneously to the assessment of health indicators (TABLE 2). Data were obtained from 30 equidistant locations, at bird level. Relative humidity, temperature and carbon dioxide concentration (CO_2) were assessed with Akso AZ 77535 (Honk Kong, China), as well as the external temperatures at the beginning and end of data collection. Air velocity, ammonia concentration (NH_3) and light intensity were measured with AK821 Akso, SP2nd NH_3 Senko Portable Single-Gas Ammonia Detector SP22N7 and Highmed Multifunctional Meter THDLA-500, respectively.

TABLE 2 – INDOOR RELATIVE HUMIDITY, TEMPERATURE, AIR VELOCITY, LIGHT INTENSITY, AMMONIA (NH_3), CARBON DIOXIDE (CO_2) AND LITTER MOISTURE ASSESSED IN TEN CLOSED-SIDED AND TEN OPEN-SIDED POULTRY HOUSES, FROM MARCH TO APRIL 2019, IN THE WEST OF SANTA CATARINA STATE, SOUTH OF BRAZIL.

Variable	Poultry houses		
	Closed-sided	Open-sided	*P*
Relative humidity (%)	74.7±13.2	72.3±11.3	0.660
Temperature (ºC)	25.9±1.8	25.9±2.2	0.995
Air velocity (m s^{-1})	2.1±0.7	1.1±1.0	<0.001
NH_3 (ppm)	11.2±6.8	7.5±3.9	0.014
CO_2 (ppm)	1,124.9±461.5	841.0±158.0	0.025
Light intensity (lx)	6.9±6.3	274.2±241.9	<0.001
Litter moisture (%)	39.5±13.1	38.6±6.4	0.422

For the litter moisture analysis, approximately 400 g of litter were collected at 12 locations per house, avoiding areas near or below the feeders or drinkers. These samples were packed in plastic bags, identified and sent for analysis at the laboratory. Following Tedesco et al. (1995) for the measurement of litter moisture, 20 to 30 g of litter samples were homogenized and placed in a forced ventilation oven for 24 or 48h, or until no change in weight was observed with increasing drying time, at 65-70°C.

2.2.3 Bird behaviour

Bird behaviour was recorded with two Canon Vixia HF R800 video cameras. Two 1.5 x 1.5 m steel cable structures were used to demarcate the bird observation area on the floor, one placed in the middle of the house and the other near the wall. The behaviour of birds that were completely visible and with more than half of their bodies within the physical structure was assessed, according to a pre-defined ethogram (TABLE 3). Observations were made during 4 h per day, for each site of the house, using scan sampling with instantaneous recording every 10 min (MARTIN; BATESON, 1993), totaling 8 h of behavioural observations per unit during the hours of day-time.

TABLE 3 - ETHOGRAM USED TO RECORD BROILER CHICKEN BEHAVIOUR IN TEN CLOSED-SIDED AND TEN OPEN-SIDED POULTRY HOUSES, FROM MARCH TO APRIL 2019, IN THE WEST OF SANTA CATARINA STATE, SOUTH OF BRAZIL.

Behaviour	Definition
Feeding	Having the head in the feeder or pecking at the feed in the feeder
Drinking	Having the beak in touch with the drinker
Foraging	Pecking and/or scratching on the floor
Exploration	Interacting with physical structures that are used to delimit the bird observation area
Comfort	Preening, wing flapping, wing stretching, feather ruffling or shaking, and elements of dustbathing behaviour
Resting	Sitting, lying, or standing while not engaged in other activities, eyes are opened or closed
Locomotion	Running, walking, or jumping
Other	Any additional behaviour performed by broiler chicken other than those included in the ethogram such as vigilance and panting. Elements of aggressive behaviour towards another broiler chicken, such as threatening, leaping, kicking, wing flapping or feather pecking, being disturbed by another bird or disturbing another bird and positive social behaviour such as allow grooming

Feeding behaviour was not assessed in nine CS and four OS houses next to the wall, and in four CS and three OS in the middle of the house due to the absence of feeders and drinkers within the physical structure. Behaviours with fewer than 20 events during the 4h-observation period were aggregated into the class “other”, except for exploration. Exploratory behaviour was affected by the assessment method, since the birds showed interest in and interacted with the physical structures.

For assessment of the human-animal relationship, a touch test was used in which the observer attempted to touch birds in 21 trials in each barn, recording the number of birds within an arm's length and the number of birds actually touched at each trial. For these results, the data was expressed as a number score that ranged from zero to 100, with zero meaning that no animals were touched, and 100 that all animals within reach touched, based on calculations in the 'Good human-animal relationship' section within the Welfare Quality® protocol (WELFARE QUALITY®, 2009).

2.2.4 Bird affective states

After a 10 min observation period, the Qualitative Behaviour Assessment (QBA) was performed before other indoor evaluation procedures were started. The assessor recorded 25 emotional descriptors on a visual analogue scale that ranged from 0 mm (indicating that the emotion seemed entirely absent in the group of animals observed) to 125 mm (the emotion seemed dominant; WELFARE QUALITY®, 2009; SOUZA et al., 2021). The terms used were the Portuguese equivalents for 'scared', 'inquisitive', 'painful', 'relaxed', 'aggressive', 'positively occupied', 'lethargic', 'comfortable', 'fearful', 'active', 'dull', 'confident', 'agitated', 'interested', 'apathetic', 'playful', 'desperate', 'apprehensive', 'attentive', 'distressed', 'calm', 'frustrated', 'lively', 'disturbed' and 'tranquil', developed for Brazilian Portuguese native speakers (SOUZA et al., 2021).

2.2.5 Statistical analysis

Differences in stocking density, house size, flock size, age at visit, age at slaughter, body weight at slaughter, mortality, culls, touch test and litter moisture according to the type of poultry house were analyzed by *t*-test for two independent samples.

For bird soiling, foot-pad dermatitis, hock burn, lameness and contact dermatitis on the breast and abdominal areas, data were fitted into a multinomial model that considered the type of house as the explanatory variable. The house effect was also incorporated into the models by means of a random effect assumed to follow a normal distribution with the mean equal to zero and constant variance (σ^2). Two classes of regression models were considered for the multinomial data, the proportional odds models and the generalized logit models. Due to the low frequencies of some indicators, scores were aggregated as follows: contact dermatitis on the breast and abdominal areas, where C1 corresponds to the 0 score, C2 = 1 and C3 = 2 + 3; for bird soiling, C1 = 0, C2 = 1 and C3 = 2 + 3; for foot-pad dermatitis, C1 = 0, C2 = 1 and C3 = 2 + 3 + 4; for hock burn, C1 = 0, C2 = 1 and C3 = 2 + 3 + 4; and for lameness, C1 = 0 + 1, C2 = 2 + 3 and C3 = 4 + 5. The likelihood ratio test was used for these five indicators to verify the assumption of proportional odds for ordinal scale data at 5% significance. The results provided by the fitted model were presented as odds ratios. The odds ratios were associated with lower scores of the indicators, meaning worse welfare, and respective confidence intervals. In addition, the estimated probabilities are also presented in plots. The Wald test, based on the asymptotic normality of the maximum likelihood estimators, was used to evaluate the effect of house type.

Data from the slaughterhouse were analyzed with generalized linear models. The half normal plot for residuals with simulated bands was used in order to detect overdispersion or any other source of lack of fit. For fractures, bruises and scratches, a binary logistic regression model was used. Furthermore, for scratches, a proportional odds regression model, for ordinal data, was used. For DOA and diseases, such as ascites, arthritis, dermatosis, myopathy and air sacculitis, a regression model with Poisson response was initially fitted; however, due to data overdispersion, the negative binomial regression model was used. The negative binomial distribution allowed for the incorporation of the additional variation present in the available data which had not been accounted for the type of house, i.e. factors specific to the poultry houses. At this stage, the only explanatory variable considered was the type of house and log corresponding to the number of animals in each poultry house.

The environmental measurements were analysed by fitting linear models, including random effects for each poultry house design. To accommodate possible heterogeneity of variances in both types of house, an additional parameter was incorporated into the model to adjust eventual heteroscedasticity between house types. The difference between the mean environmental conditions of houses was tested based on the student’s *t*-test distribution.

Data from the animal behaviour assessment were analyzed by fitting regression models to count data. The frequencies of the different types of behaviour were analysed through log-linear models, as usually applied to data available on multi-dimensional contingency tables. In such cases, the registered frequencies are taken as the response variable, and all categorical variables composing the contingence table are considered predictors. The effect of type of house on type of behaviour was assessed by testing their corresponding interaction. The effect of recording location, in the side- or mid-location in the barn, was considered also. As not all observed areas included feeder and drinker records, a possible effect of access or otherwise to feeders and drinkers was included in the fitting model by means of an indicator covariate. Finally, the total log frequencies of animals in each poultry house were included in the model. The data were firstly analysed using the Poisson distribution. However, as the data again showed overdispersion, we opted for the negative binomial distribution, with a logarithm link function. In the case of multiple comparisons, the *P*-values were adjusted using Tukey's method.

Principal component analysis (PCA; JOHNSON; WICHERN, 2007) was conducted, with no rotation, in order to exploit the correlation structure of the 25 investigated features for QBA. Parallel analysis (FRANKLIN et al., 1995), based on simulated datasets under independence structure, was used to choose how many components to retain. Two components explained most of the variance in the data. With the results from PCA, the principal co-ordinates (scores) for each type of house were calculated and then the comparison of the scores for CS and OS houses were performed. The difference between house types was tested based on the *t*-test for two independent samples for each component.

All conclusions were based on a significance level of 5%, using R software (R CORE TEAM 2019). The ordinal package was used to fit multinomial models, nlme package for mixed linear models, and the ggplot2 package for graphics.

2.2.6 Ethical approval

This project was approved by the Animal Use Ethics Committee of the Agricultural Campus (No 046/2018), of the Federal University of Paraná (ANNEX I, II).

2.3 RESULTS

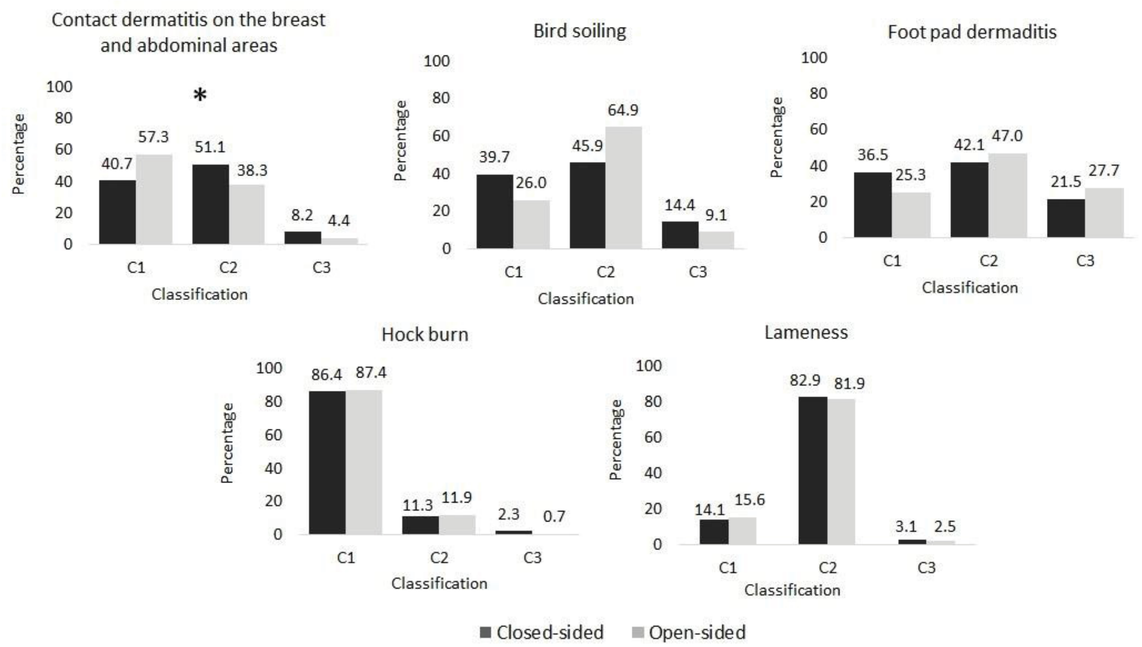
2.3.1 Health assessment

The only health indicator assessed on farm that differed between CS and OS houses was contact dermatitis on the breast and abdominal areas, with better scores in OS houses (TABLE 4, FIGURE 2).

TABLE 4 - ESTIMATED ODDS RATIOS FOR WORSE SCORES ON CONTACT DERMATITIS ON THE BREAST AND ABDOMINAL AREAS, BIRD SOILING, FOOT-PAD DERMATITIS, HOCK BURN AND LAMENESS FOR TEN CLOSED-SIDED AND TEN OPEN-SIDED POULTRY HOUSES, FROM MARCH TO APRIL 2019, IN THE WEST OF SANTA CATARINA STATE, SOUTH OF BRAZIL.

Variables	Closed- / Open-sided poultry houses		
	Odds ratio	CI (95%)	*P*
Contact dermatitis on the breast and abdominal areas	2.16	(1.10; 4.28)	0.026
Bird soiling	0.71	(0.16; 3.06)	0.651
Foot-pad dermatitis	0.60	(0.15; 2.32)	0.467
Hock burn	0.83	(0.15; 2.32)	0.744
Lameness	1.10	(0.46; 2.63)	0.821

FIGURE 2 - OVERALL MEAN PERCENTAGE OF CONTACT DERMATITIS ON THE BREAST AND ABDOMINAL AREAS (C1 CORRESPONDS TO THE 0 SCORE, C2 = 1, AND C3 = 2 + 3); BIRD SOILING (C1 = 0, C2 = 1, C3 = 2 + 3); FOOT-PAD DERMATITIS (C1 = 0, C2 = 1, C3 = 2 + 3 + 4); HOCK BURN (C1 = 0, C2 = 1, C3 = 2 + 3 + 4); AND LAMENESS (C1 = 0 + 1, C2 = 2 + 3, C3 = 4 + 5); *P<0.05 DENOTES A SIGNIFICANT DIFFERENCE.



The average DOA was 0.05 (±0.02)% and 0.04 (±0.02)% for CS and OS houses, respectively. The only slaughterhouse health indicator that differed between house types was scratches. The odds ratio OS/CS houses estimated for this lesion was 1.29 (P = 0.043). Means of 59.5 and 66.8% of some level of scratches (score 1 to 3) were observed in CS (0 score = 40.5%, 1 = 39.3%, 2 = 16.5% and 3 = 3.8%) and OS (0 score = 33.2%, 1 = 44.0%, 2 = 16.0% and 3 = 6.8%) houses, respectively. And finally, the frequencies of occurrence of fractures were 0.005 and 0.01% and of bruising were 0.18 and 0.14% for CS and OS houses, respectively.

2.3.2 Bird behaviour

Two behaviours presented different frequencies between CS and OS houses (TABLE 5). The odds ratio of exploratory behaviour was 75.1% higher (1.75 times) in OS compared to CS houses; for category “other” the odds ratio was 87.7% higher (1.87 times). Within the “other” category, the main behaviour was panting (97.6%), with frequencies of 93.1% in CS and 97.4% in OS houses. The frequencies of drinking (P = 0.610) and feeding (P = 0.380) showed no significant difference between CS and OS houses.

TABLE 5 - RELATIVE FREQUENCIES OF BEHAVIOURS ACCORDING TO TEN OPEN-SIDED RELATIVE TO TEN CLOSED-SIDED POULTRY HOUSES, FROM MARCH TO APRIL 2019, IN THE WEST OF SANTA CATARINA STATE, SOUTH OF BRAZIL.

Behaviour	Ratio	SE	*P*
Foraging	0.76	0.15	0.198
Exploration	1.75	0.39	0.012
Comfort	1.11	0.22	0.603
Resting	0.91	0.17	0.638
Locomotion	1.17	0.24	0.427
Other	1.87	0.37	0.002

Overall, there was a mean of 25.0 (±7.0) birds within the physical structure for behavioural observation next to the wall and 29.6 (±6.6) birds within for the structure in the middle of the house; the same trend was observed for both house types. In both types of house and all observation sites, most birds (55.0%) exhibited resting behaviour. This behaviour accounted for 59.5% of total behavioural activities in CS and 50.5% in OS houses, followed by “other” (9.0 and 16.2%), comfort (9.4 and 10.2%) and foraging (7.2 and 4.8%) behaviours, respectively.

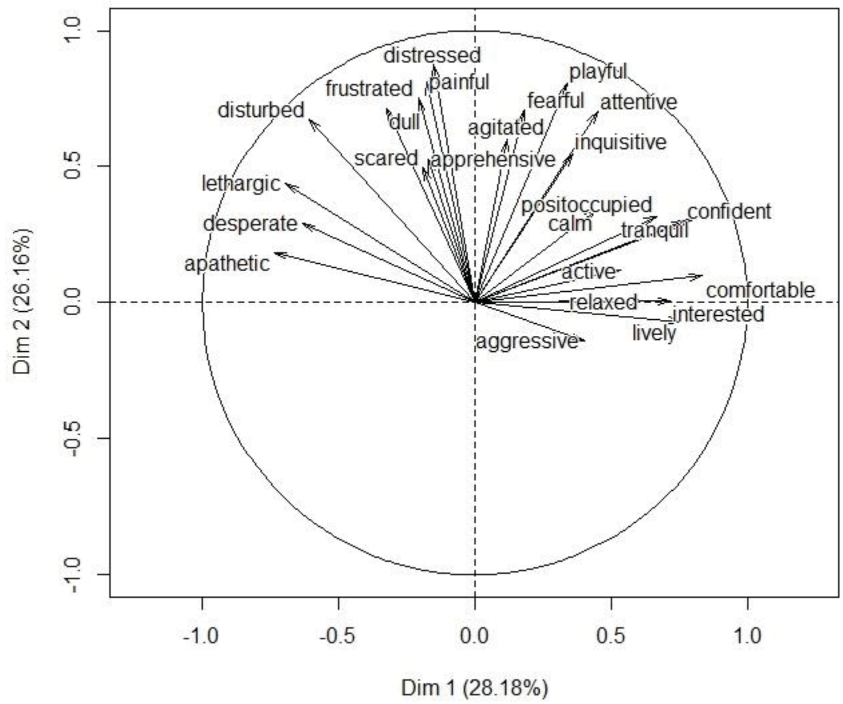
The touch test presented high mean scores (min-max) of 90 (71-100) in CS and 86 (70-99) in OS houses ($P = 0.179$). The mean number of birds within arm's reach per attempt was 2.8 (±2.0) birds in CS and 2.3 (±1.8) birds in OS houses; the number of broiler chickens actually touched was 3.0 (±1.0) and 2.0 (±1.0) chickens for CS and OS houses, respectively.

2.3.3 Bird affective states

Principal component analysis of the 25 QBA terms revealed two principal components which explained 28.18 and 26.16% of the variation. Scores for the first and second components presented no difference between CS and OS houses. The average scores and standard deviations for the first component were 0.75 (±0.72) and -0.75 (±3.46; $P = 0.227$), and in second component -0.95 (±3.33) and 0.95 (±2.99; $P =$

0.118), for CS and OS houses, respectively. FIGURE 3 shows the overall component loadings of each QBA term across the two principal components. The first component suggests a mood dimension, with higher loadings representing positive emotions that ranged from playful to comfortable and lower loadings ranging from painful to apathetic. The second component ranged from distressed to aggressive.

FIGURE 3 - PRINCIPAL COMPONENT LOADINGS FOR EACH QUALITATIVE BEHAVIOUR ASSESSMENT TERMS ACROSS THE TWO PRINCIPAL COMPONENTS, FOR TEN CLOSED- AND TEN OPEN-SIDED POULTRY HOUSES, FROM MARCH TO APRIL 2019, IN THE WEST OF SANTA CATARINA STATE, SOUTH OF BRAZIL.



2.4 DISCUSSION

Our aim was to assess the effect of CS and OS house designs on broiler chicken welfare indicators. Results obtained in CS houses were worse for environmental measures, such as light intensity, NH_3 and CO_2 concentrations, and two animal-based measures, contact dermatitis on the breast and abdominal areas, and exploratory behaviour. The higher stocking density practiced in CS houses, as described in the literature (TUYTTENS et al., 2015; LIMA et al., 2020) was confirmed. The animal density results are relevant also because citizens perceive stocking density and pen sizes as very essential for farm animal welfare (VANHONACKER et al., 2009). For OS houses, we observed slower air velocity as well as higher prevalences for two animal-based measures, namely scratches and panting behaviour. Other house effects on health and environmental indicators, bird behaviour and affective states were not observed.

It is important to consider that animal welfare may be understood in different ways. For the World Organisation for Animal Health (OIE, 2019a), the scientific assessment of animal welfare involves diverse elements which need to be considered together; selecting and weighing these elements often involves value-based assumptions. Thus, the OIE (2019b) recommended some useful indicators of broiler chicken welfare, such as mortality, gait, contact dermatitis, feather condition, incidence of diseases, metabolic disorders, behaviour, water and feed consumption, performance, biosecurity, and animal health, that may be adapted to the different situations where these birds are managed, and most of these indicators were assessed in this study. Birds reared in CS houses were 2.16 times more likely to have contact dermatitis on the breast and abdominal area as compared to those reared in OS houses (TABLE 4). Contact dermatitis is an important animal-based indicator, and both hock burn and foot-pad dermatitis are associated with pain (EFSA, 2012b). Besides, further evidence for the importance of dermatitis has been proposed by Souza et al. (2015b), who observed absence of breast blister, the former indicator for this area of the body, in both certified and non-certified intensive poultry farms in the State of Paraná, Brazil, suggesting that a more sensitive indicator was needed. Therefore, the assessment of contact dermatitis on the breast and abdominal areas was developed and tested (SOUZA et al., 2018b); this indicator was clearly useful in distinguishing bird welfare between two different types of houses in our work. Different factors may affect the prevalence of contact dermatitis. Although re-using litter is common practice in the Brazilian poultry industry (CARVALHO et al., 2011), this may lead to lesions and compromise broiler chicken welfare (BARACHO et al., 2013) as it relates to lower litter quality for animals raised in re-used bedding.

When moisture values are higher than 30% litter may be considered wet, and this litter condition has been associated with dermatitis (TAIRA et al., 2014); this value is close to those in both types of poultry house studied. The number of flocks per litter and the stocking density, both higher in CS than OS houses, are associated with higher litter moisture and may have contributed to decreased litter quality, which is considered an important factor in the appearance of skin lesions (ALLAIN et al., 2009). In general, higher stocking densities are associated with several animal health and behaviour problems, as well as poor litter quality (BUIJS et al., 2009; EFSA, 2012a; LIMA et al., 2020). Bailie, Ijichi, O'Connell (2018) also suggest that increasing stocking density is a risk factor for more severe dermatitis. Thus, litter quality seems relevant for bird welfare and the monitoring and corrections for environmental quality may prevent its negative consequences for the animals.

Scratches were more prevalent in OS than CS houses. Allain et al. (2009) evaluated various types of lesions in broiler chicken flocks in France and observed most of the flocks (48/55) with scratches, a prevalence equivalent to 79.7 (±13.1)%, a value which is higher than our results. Souza et al. (2018b) assumed the multiple occurrences of the same type of lesions as being indicative of a welfare problem and increased suffering. The higher the automation level of the house, the lower the incidence of scratches (PILECCO et al., 2011a), thereby providing a general rationale for the lower occurrence of scratches in CS than OS houses. However, this rationale does not clarify the underlying causes for the lesions. Increased stocking density, lack of plumage, type of daily handling, age and gender of the bird, catching procedures, number of birds per transport box, transport quality and duration, and number of hours that birds await slaughter may all be considered as potential risk factors for scratches (ELFADIL; VAILLANCOURT; MEEK, 1996; PILECCO et al., 2011a; PILECCO et al., 2011b). The light intensity, one of the significant differences between CS and OS houses, may be related to the greater occurrence of scratches in OS, since a better lit environment tends to increase bird activity and this, in turn, may result in more scratches. On the other hand, an environment with low lighting, as was permanently the case in CS houses, may minimise fear reactions in birds (HFAC, 2014). However, it may not be possible to sufficiently reduce lighting in OS houses during catching procedures. This situation may be considered a critical point for animal welfare, due to the increased prevalence of scratches it may cause. In addition, according to Bailie, Ball, O'Connell (2013), the increased contrast between lighter and darker areas may increase bird's perception of items which are relevant to them. This information suggests that the birds perceive and better manage their environment when exposed to important environmental conditions, such as adequate lighting. This hypothesis also seems in accordance with the greater occurrence of exploratory behaviour observed in OS houses. However, the possible causes for the scratches require further investigation and the development of strategies for their avoidance, especially because scratches are painful to the birds.

Overall results regarding relative air humidity (TABLE 2) were close to acceptable limit of 70% (EFSA, 2012a; DEFRA, 2018). Both CS and OS houses presented average temperatures higher than the 21-22º C (TABLE 2) recommended for 6 week old broiler chickens (FURLAN; MACARI, 2002). Even though comfortable temperatures are more expected in CS when compared to OS houses (CARVALHO et al., 2015), our results regarding summer and autumn did not confirm this expectation and birds in both house types were subjected to thermal discomfort. This situation is likely related to the fact that the welfare assessment was conducted during the summer, and more research is needed to understand whether results from these two types of housing differ during other seasons. Overall results for panting behaviour showed high frequencies in both type of houses, and significantly higher values for birds in OS houses. Federici et al. (2016) observed median scores for thermal comfort, classified by the Welfare Quality® protocol as acceptable, in OS houses with extra fans and with high frequency of panting. However, the same authors emphasised that the increase in use of CS houses may not solve the problem of heat stress, because of the higher stocking densities commonly practiced in CS houses as compared to OS houses in Brazil. The excessive heat is a highly stressful factor for birds (OLANREWAJU et al., 2010), which emphasises the importance of controlling thermal stress in both types of houses. Our results for panting may be associated with the barn ventilation rates, which were different between CS and OS houses (TABLE 2). The ventilation may help to remove moisture and heat, promoting air renewal (NÄÄS et al., 2014). Therefore, both panting and ventilation require monitoring, preferably by closely verifying animal-based indicators such as panting.

Although the concentrations of NH_3 and CO_2 did not exceed the respective limits of 20 and 3,000 ppm (EU, 2007; RSPCA, 2017; DEFRA, 2018) in any type of house, CS houses showed higher concentrations of these gases. Probably the handling of the curtains favoured air renewal in OS houses, even though at the time of the assessment the air velocity was 0 m s^{-1} in 64/300 measurements in OS houses, whereas in CS houses air velocity was never lower than 0.5 m s^{-1}, the minimum recommended for broiler chickens after 14 days of age (COBB, 2018). Ventilation and air quality are recognised as key factors for animal welfare (JONES; DONNELLY; DAWKINS, 2005; BARACHO et al., 2018). Stocking density is also an important factor along with environmental indicators (JONES; DONNELLY; DAWKINS, 2005). Our results show that indoor environmental indicators need improvement in both poultry house types. This may be achieved by reducing the production of harmful gases, with strategies involving the reduction of stocking densities, improvement in litter quality and providing higher air renewal. In addition, our results indicate the need for managers of both types of poultry house to monitor more closely and take corrective actions for indoor air quality and velocity.

Different light intensity values were observed between house types. The CS houses (6.9 [± 6.3] lx) were far below the broiler chicken welfare recommendations of a minimum of 20 lx measured at bird eye level (EU, 2007; EFSA, 2012a), even though it complies with the recommendations from the breeder companies of 5-10 lx (ROSS, 2014; COBB, 2018). Clearly private recommendations that are below regulatory animal welfare thresholds constitute an important problem to be addressed. Birds reared in 5 lx are less active than those in 20 lx (RAULT et al., 2016). Additionally, under 1 lx, fundamental eye characteristics such as eye size are affected (DEEP et al., 2010). Nonetheless, in CS houses very low light intensity was used for at least 60% of the bird's lives, which may force a constant resting state on broiler chickens. According to Paranhos da Costa, Lima, Sant'anna (2017), bird behaviour under continuous low lighting may be confounded with a calm state; however, animals may be in an apathetic state instead. Our results did show that light intensity was, on average, much lower in CS, which may be aggravated by a lack of standardisation of the provided light types. Light characteristics may directly influence physical, psychological and behavioural aspects of chicken welfare. For instance, some light sources provide light without emitting relevant ultraviolet wavelengths (BAILIE; BALL; O'CONNELL, 2013), which impair the visual capabilities of chickens, that differ from human visual abilities (PRESCOTT; WHATES; JARVIS, 2003). Therefore, new studies into the types of lighting used in commercial farms and their welfare consequences are warranted.

Weary (2014) suggests behaviour assessment as a method of identifying animal suffering, to observe if an animal is experiencing a negative affect such as pain, as animals tend to show a decline in highly motivated behaviours when in negative emotional states. We have observed a high prevalence of some lesions and a restricted behavioural repertoire. Statistical differences between birds from CS and OS houses were observed for exploratory behaviour and the “other” category, mostly composed of panting. Classically, exploratory behaviour relates to the search for information about the environment. From an evolutionary perspective, it was probably important for birds to anticipate and seek changes through exploratory behaviour; however, the paucity of stimuli may lead the animals to decrease their motivation to explore (NEWBERRY, 1999). This information suggests that the OS houses may offer better conditions for birds than CS in terms of exploratory behaviour, as the broiler chickens, when motivated, may be more able to seek opportunities to explore novel stimuli (NEWBERRY, 1999). According to our observations, the physical structure used to delimit the experimental bird observation area served to promote exploration in both types of house, and the difference in exploratory behaviour may be related to the higher light intensity in OS. Birds reared in OS houses showed higher panting behaviour than those in CS houses, suggesting that OS houses require improvements regarding indoor temperature control. Although the OS houses may lead to better air quality and more behavioural opportunities for the birds, there is a risk for animals suffering due to exposure to high temperatures (LIMA; SILVA, 2019), which was evident in our results.

Resting was the commonest behaviour, which may be related to bird age and locomotor problems. In a study by Weeks et al. (2000), birds aged between 39 and 49 days of age remained lying on average 76% of the time, and this percentage increased to 86% for birds with score 3 for lameness, described as a bird with obvious gait abnormality that affects the ability to move. In our study, the mean resting time was 55.0% and lameness scores 2 + 3 showed high percentages in both CS (82.9%) and OS houses (81.9%). Lack of environmental complexity may also be a cause of high frequencies of resting behaviour. According to Bailie, Ball, O'Connell (2013), birds may engage in other activities if stimulated. During our data collection, exploratory behaviour, which is considered important for the birds (NEWBERRY, 1999), differed statistically between CS and OS houses. No environmental enrichment was available for the birds, emphasising that the industry is still very limited in relation to the consideration of birds’ behavioural needs.

Results from QBA, which considers the expressive quality of how animals behave and interact with the environment and with each other (WELFARE QUALITY®, 2009), did not reveal differences according to house types and the set of terms displayed by first and second components seemed consistent between house types. For example, flocks with emotional states such as comfortable and tranquil did not express desperate or apathetic states, being observed in opposite directions. On the other hand, flocks in painful or distressed moods were also associated with fearful or agitated feelings. However, Tuyttens et al. (2015) showed differences between broiler flocks assessed in Belgium, in CS houses and in Brazil, in OS houses. The authors observed Brazilian flocks as more comfortable, content, energetic and positively occupied than Belgian flocks. Therefore, greater understanding of the effects of house type on positive emotional states may benefit from further research.

The touch test relies on the rationale that broiler chickens will withdraw from the observer if they are fearful (WELFARE QUALITY®, 2009). Our results showed high mean scores (90 in CS and 86 in OS houses) in both types of poultry houses, indicating few avoidance reactions towards humans. However, the results of this test may also be associated with reduced walking ability, when birds have more difficulty in reaching valued resources or expressing emotional states (VASDAL et al., 2017). Our results for the touch test may be related to the prevalences of more severe lameness scores (3 and 4), which were 3.1 and 2.5% in CS and OS houses, respectively. These percentages were lower than that the 14.0% observed by Federici et al. (2016) for 4 and 5 scores, in a study with a score of 99 for touch test. Thus, data considering lameness scores and touch test suggest that the higher the prevalence of severe lameness, the more birds are touched, indicating that the intuitive positive correlation between lameness and touch test may be correct and that the idea of the touch test as a measure of fear should be challenged. Additionally, although our results did not differ between types of poultry houses, Bassler et al. (2013) found that length of dark period for broiler chickens at three weeks of age was a risk factor for the touch test results for 89 flocks assessed. Thus, it is also possible that the touch test results may differ according to lighting programs (FEDERICI et al., 2016). Overall, our touch test results endorse the perceived flaws regarding its value as a measurement of bird fear of humans.

2.5 CONCLUSIONS

Closed-sided poultry houses showed worse welfare results considering environmental indicators such as light intensity, NH_3 and CO_2 concentrations, and for two animal-based measures, namely contact dermatitis on the breast and abdominal areas and exploratory behaviour. Air velocity and two other animal-based measures namely scratches and behaviours classified as “others”, mostly composed of panting, showed worse results for open-sided houses. There were no other significant differences between both housing types on health assessment, environmental measurements, bird behaviour or affective states. This research has revealed that bird welfare in both house types, for the region and season assessed, was compromised as evidenced by poor environmental conditions, considerable behavioural restrictions and a high prevalence of injuries.

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3. WELFARE OF BROILER CHICKENS REARED IN TWO DIFFERENT INDUSTRIAL HOUSE TYPES DURING THE WINTER SEASON IN SOUTHERN BRAZIL

RESUMO

Comparamos o bem-estar de frangos de corte mantidos em sistemas intensivos e em dois tipos de galpão, dark-house (CS) *vs.* semi-climatizado (OS), durante a estação do inverno, no Sul do Brasil. Foram avaliados 10 lotes de cada tipo de galpão, para os seguintes indicadores: a) indicadores de saúde: dermatite de contato nas áreas do peito e abdômen (CDE), limpeza das aves (BSO), pododermatite (FPD), queimadura de jarrete (HBU), claudicação (LAM), fraturas (FRA), hematomas (BRU), arranhões (SCR), aves mortas no transporte (DOA), e doenças (DIS); b) indicadores ambientais: umidade relativa (RHU), temperatura (TEM), velocidade do ar (AVE), iluminância (ILL), amônia (NH_3) e dióxido de carbono (CO_2); c) indicadores comportamentais e estados afetivos: comportamento da ave (BBE), teste do toque (TTE), e avaliação qualitativa comportamental (QBA). A análise estatística foi baseada em um modelo de regressão para CDE, BSO, FPD, HBU, LAM e modelos lineares generalizados para DOA, FRA, BRU, SCR e DIS. O teste de Mann-Whitney foi usado para RHU, TEM, AVE, ILL, NH_3, CO_2, e o teste T para TTE e LMO, com um modelo de regressão específico para BBE e Análise de Componentes Principais para QBA. De acordo com as chances dos piores escores observados em CS em relação à OS, as aves foram menos propensas a apresentar escores severos de CDE (P=0,040 e P=0,007), BSO (P=0,031; P=0,016; P=0,038), e HBU (P=0,017), e apresentaram valores medianos mais altos para AVE (2,3, 0,0-7,8 m s^{-1} *vs*. 0,0, 0,0-4,3 m s^{-1}), menor concentração de NH_3 (9,0, 0,0-64,0 ppm *vs*. 12,0, 0,0-60,0 ppm) e para os escores de TTE (98, 96-100 *vs*. 67, 25-100). Foram observados piores resultados em galpões CS para densidade (13,8±0,2 aves/m^2 *vs*. 12,0±0,2 aves/m^2), RHU (74,5, 50,7-99,9% *vs.* 72,3, 47,4-99,9%), e TEM (23,9, 14,6-29,2ºC *vs.* 21,7, 12,9-30,1ºC), menor ILL (16,0, 1,0-60,0 lx *vs*. 161,0, 8,0-2380,0 lx), menor expressão do comportamento de beber (P=0,007), aves mais inativas (P<0,001), e menor observação de emoções positivas de acordo com o QBA (P=0,028). Os resultados observados na região e estação do ano estudadas indicaram que os galpões CS parecem oferecer menos problemas de bem-estar em relação aos indicadores de saúde; entretanto, os galpões do tipo OS mostraram menor restrição em relação a expressão comportamental e estados emocionais positivos.

Palavras-chave: abate, ambiente, aviário, avicultura, comportamento, estados emocionais, saúde.

ABSTRACT

The following trial compared broiler chicken welfare in closed-sided (CS) *vs.* open-sided (OS) industrial house types during the winter season in the South of Brazil. Ten flocks in each house type were evaluated as follows: a) bird health: contact dermatitis on the breast and abdominal areas (CDE), bird soiling (BSO), footpad dermatitis (FPD), hock burn (HBU), lameness (LAM), fractures (FRA), bruising (BRU), scratches (SCR), dead on arrival (DOA), and diseases (DIS); b) house environmental measurements: relative humidity (RHU), temperature (TEM), air velocity (AVE), illuminance (ILL), ammonia (NH_3) and carbon dioxide concentration (CO_2); c) bird behaviour and affective states: bird behaviour (BBE), touch test (TTE), and qualitative behaviour assessment (QBA). Statistical analyses were based on regression models for CDE, BSO, FPD, HBU, LAM and generalised linear models for DOA, FRA, BRU, SCR and DIS. The Mann-Whitney test was used for RHU, TEM, AVE, ILL, NH_3, CO_2, and the t-test for TTE and LMO, with a specific regression model for BBE data and Principal Component Analysis for QBA. According to odds ratio for worse scores for CS relative to OS, birds were less likely to have severe scores for CDE (P=0.040 and P=0.007), BSO (P=0.031, P=0.016, and P=0.038), and HBU (P=0.017), and had higher median values for AVE (2.3, 0.0-7.8 m s^{-1} *vs*. 0.0, 0.0-4.3 m s^{-1}), lower NH_3 concentration (9.0, 0.0-64.0 ppm *vs.* 12.0, 0.0-60.0 ppm) and TTE scores (98, 96-100 *vs*. 67, 25-100). Worse results were observed in CS houses for higher stocking density (13.8±0.2 birds/m^2 *vs*. 12.0±0.2 birds/m^2), RHU (74.5, 50.7-99.9% *vs.* 72.3, 47.4-99.9%), and TEM (23.9, 14.6-29.2ºC *vs.* 21.7, 12.9-30.1ºC), lower ILL (16.0, 1.0-60.0 lx *vs*. 161.0, 8.0-2380.0 lx), less drinking (P=0.007), more inactive behaviour (P<0.001) and lower positive emotions, according to QBA (P=0.028). In the studied region and season, CS houses seemed to offer fewer welfare problems in terms of the health indicators; however, OS houses showed fewer behavioural restrictions and higher positive emotional states.

Keywords: aviary, behaviour, emotional state, environment, health, poultry, slaughter.

3.1 INTRODUCTION

Animal welfare is a current and increasingly relevant issue in animal production. The importance of this is growing due to greater awareness of both markets and consumers around the world which demand more ethical production systems which are less harmful to animals (EU, 2015; WAP, 2016; QUEIROZ et al., 2018; ALONSO; GONZÁLEZ-MONTAÑA; LOMILLOS, 2020).

The poultry meat industry involves a large number of chickens worldwide therefore, there is a high priority for improving animal welfare in this context (ROWE; DAWKINS; GEBHARDT-HENRICH, 2019). In 2019, Brazil was the third largest producer of poultry meat with 13.2 million tons, after the United States of America with 19.9 million tons and China with 13.7 million tons. Brazil was the number one exporter (4.2 million tons; ABPA, 2020). This same year, the Brazilian industry sent around 5.8 billion broiler chickens to slaughter, with the State of Paraná being the highest in numbers of slaughtered animals and Santa Catarina, where this study was conducted, as the second highest, the latter being equivalent to 15.4% of national total (ABPA, 2020; IBGE, 2020).

Brazilian poultry production is distributed across all regions of Brazil, and different types of barn designs are employed as farm units, which may be categorised as conventional, semi- and fully acclimatised houses. The conventional and semi-climatised barns are generally characterised by open sides with curtains and artificial or natural light (or both). Of these two house types, only the semi-climatised barns are equipped with positive pressure fans and sprinklers. The Brazilian climatised barn is characterised by artificial lighting, negative pressure and evaporative cooling systems, exhaust fans and sprinklers, and fully curtain-closed sides. Curtains may be double and have different colours, such as black, blue, green or yellow (BARACHO et al., 2018; LIMA; SILVA, 2019). Although the reason for using closed-sided barns is to provide better control of internal environmental conditions, open-sided barns may present better air quality due natural ventilation (LIMA et al., 2020; SANS et al., 2021). Likewise, the absence of natural light in closed-sided barns can limit behavioural expression in birds (BAILIE; IJICHI; O'CONNELL, 2018; LIMA; SILVA, 2019). On the other hand, conventional and semi-climatised poultry houses may increase bird suffering due to thermal stress (LIMA; SILVA, 2019). Thus, it is important to study which is house design fosters a positive association between animal welfare and environmental conditions (MAZZUCO; SILVA; ABREU, 2019), through the monitoring of animal welfare indicators.

The assessment of animal welfare involves diverse elements which, when considered together, contribute to an understanding of issues in each production system. Some measures of animal welfare assess the degree of impaired functioning associated with injury, disease and malnutrition, others focus on animal needs and affective states, and some assess the physiological, behavioural and immunological changes that animals show in response to various challenges (OIE, 2019a). Some welfare indicators for broiler chickens have been consolidated by international protocols and recommendations which may be assessed for every life stage of the birds (WELFARE QUALITY, 2009; OIE, 2019b). The major critical points are well known and revolve around problems with locomotor activity, mortality, culling, morbidity, skin lesions, thermal discomfort, decreased bird behavioural repertoire, barren environment and negative emotional states. These may be related to fast growth rate or inadequate internal barn conditions, in terms of ammonia, dust, relative humidity, temperature, stocking density, litter and light deprivation (BESSEI, 2006; SANS et al., 2014; SOUZA; MOLENTO, 2015; FEDERICI et al., 2016; SOUZA et al., 2018a; 2018b; OIE, 2019b). Thus, studies considering different stages of the production cycle are important to verify types of facilities and their consequences, in terms of welfare, due to climatic, cultural and economic conditions of the region (ABREU; ABREU, 2011). Although critical bird welfare points used in different production systems have been studied for decades, these require more effective solutions (GRANDIN, 2018; REIS; MOLENTO, 2019), which may be reached through regulation, continuous investment in research, dissemination of information to consumers, and, consequently, increased demand and availability of welfare-friendly products (SOUZA; MOLENTO, 2015; FRANCO et al., 2018). These solutions rely on scientific knowledge regarding the impact of management and facility choices on welfare.

The impact of closed-sided *vs.* open-sided barns on bird welfare has been studied before (SANS et al., 2021), however, results have referred only to conditions during summer and autumn, and so far, it is unknown whether these results may be generalised to other seasons. Animals reared under intensive indoor conditions, in theory, are vulnerable to meteorological conditions and the effects of climate change.

However, that may depend on the system employed, equipment available to control internal climate, as well as, to some degree, outdoor climate conditions. In case of production systems with outdoor access, shelter availability, rainfall, radiation and wind speed may influence the number of birds using the outside range (STADIG et al., 2017). In addition, different barn types may provide different levels of protection from the outside meteorological conditions, and it is possible to observe seasonal patterns regarding animal welfare aspects, i.e. footpad dermatitis and cellulitis (PART et al., 2016). Thus, climatic conditions remain important for animal welfare, both in free-range and intensive systems. It is relevant to consider meteorological factors for each geographical region because it allows for the best planning of the poultry house, in terms of their project, cardinal orientation, adequate handling of the internal environmental conditions, which all may reduce costs, and improve facilities and environmental conditions for the birds (ABREU; ABREU, 2011; PAULINO et al., 2019).

Brazil, due to its large land mass, is classified into three climate types: tropical, equivalent to 81.4%; semi-arid, to 4.9%; and subtropical, to 13.7% of national territory. The latter one has the most marked seasonal differences across summer and winter, with temperatures ranging from <10 to 22°C (ALVARES et al., 2013), and this region was used in the present study.

As previous results referred to summer and autumn conditions (SANS et al., 2021), the current research assessed the effect of common closed-sided (CS) and open-sided (OS) poultry house types on broiler chicken's welfare in Southern Brazilian conditions during the winter season.

3.2 MATERIAL AND METHODS

3.2.1 Bird husbandry

The 20 participant farms were selected according to bird age, presence of either open or closed houses with either semi- or fully climatised internal conditions, the latter with black curtains and exclusive use of artificial lighting. From July to August 2019, in the West of the State of Santa Catarina, South of Brazil, 10 closed-sided (CS) and 10 open-sided (OS) poultry houses from the same company were visited to assess bird welfare. External environmental conditions were similar, and the median temperature was 20.3ºC and 17.7°C (P=0.9681), relative humidity 70.3% and 64.6% (P=0.5352), air velocity 0.9 m s^{-1} and 0.9 m s^{-1} (P=0.5485), and illuminance 5500 lx and 7952 lx

(P=0.2301), for the CS and OS houses, respectively, as measured outside the barns during visits. Information such as initial number of birds, numbers during the visit, age, breed, mortality and culling rates were collected. The first author, an animal scientist experienced in poultry welfare and trained since 2011 in the use of the Welfare Quality® protocol for poultry, performed all on-farm assessments.

The participant farms raised male Cobb MX (five CS and four OS houses) and Ross TM4 (five CS and six OS houses), and operated in an integrated system. The birds ranged from 33 to 36 days of age, which was equivalent to 5.1±2.1 d before slaughter. A summary of the main characteristics of the farms and indicators are shown in TABLE 6.

TABLE 6 - MAIN CHARACTERISTICS OF 10 CLOSED-SIDED AND 10 OPEN-SIDED POULTRY HOUSES ASSESSED FROM JULY TO AUGUST 2019, IN THE WEST OF SANTA CATARINA STATE, SOUTH OF BRAZIL.

Variable	Closed-sided houses (n=10)	Open-sided houses (n=10)	P
Stocking density, birds/m^2	13.8±0.2	12.0±0.2	<0.001
House size, m^2	3810.0±775.2	2520.0±473.3	0.002
Flock size, number of birds at visit	49919.7±9969.4	28809.8±5654.9	<0.001
Age at visit, d	34.9±1.0	34.9±1.2	0.843
Age at slaughter, d	39.3±1.5	40.6±2.1	0.160
Body weight at slaughter, kg	2.9±0.1	2.8±0.1	0.688
Mortality (%)	3.0±1.9	3.3±1.5	0.737
Culls (%)	2.0±1.5	1.2±0.7	0.197
Reused litter (number of flocks/litter)	6.2±2.6	8.5±10.1	0.248

All CS houses had double black and silver curtains fixed to partial walls and were equipped with negative ventilation, exhaust fans, sprinklers, illuminance controllers, heating system with automatic control and air inlets. The OS houses also had partial walls, with wire mesh covered by double yellow (eight OS houses) and blue (two OS houses) roll-up curtains, positive ventilation with fans, sprinklers and both natural and artificial lighting. In general, the curtains in OS houses were half- or almost totally closed, at the time of the visits. All farmers used wood shaving as litter, automatic feeders and LED, incandescent, fluorescent or both lighting sources in the same house (FIGURE 4).

FIGURE 4 - VIEW OF CLOSED-SIDED (A) AND OPEN-SIDED (B) POULTRY HOUSES IN THE WEST OF SANTA CATARINA STATE, SOUTH OF BRAZIL.

To optimise the data collection time, in 9 out of 10 CS and all OS farms, where more than one poultry house with comparable conditions were maintained, behavioural data were recorded in one house, while other animal welfare indicators were collected in another. This allowed for the assessment of all animal welfare indicators in a single day, instead of the two days needed when only one barn was used per farm. In the CS farm with only one house used in the study, data collection started with behavioural observations followed by the welfare assessment. As a result, a total of 39 houses were evaluated, giving complete data for 20 farms.

3.2.2 Health assessment

The mean duration for bird health assessment was 292±52 min per flock. The visits and respective welfare assessments were performed between 08:15 and 16:20 hours. The on-farm health indicators included contact dermatitis on the breast and abdominal areas, and bird soiling (dirty on the body, skin or feathers birds, as defined by SOUZA et al., 2018a) both from 0 (absence) to 3 (severe), footpad dermatitis and hock burn, both from 0 (absence) to 4 (severe), assessed on a same sample of 150 birds per flock (WELFARE QUALITY® 2009; SOUZA et al., 2018a). Lameness was assessed in another sample of 150 birds, from 0 (normal gait) to 5 (unable to walk; WELFARE QUALITY®, 2009). All these assessments were performed throughout the house, which was divided into 30 equidistant locations, with 10 randomly selected birds per location, totalling 300 birds per flock.

Health indicators were collected at the slaughterhouse but with a smaller sample size *viz.* seven out of the ten CS and nine of the ten OS houses, due to assessor availability constraints. All these flocks were slaughtered in the same slaughterhouse. Two assessors, both with previous experience in collecting animal welfare data at slaughterhouses, were responsible for this phase. For harmonisation of procedures, the assessors were trained in lesion classification for fractures, bruising and scratch scores. To accommodate the high-speed line, selected carcases were assessed according to the colour of the hanging hook, which was randomly selected. In the slaughterhouse, hooks have an interspace between same coloured hooks of, on average, 10 birds or five seconds. This skipping method allowed the assessment to be performed at a slower pace as compared to line speed (SOUZA et al., 2018a).

Due to the speed of the slaughter line and the complexity of some indicators, the observer assessed one indicator at a time. A total of 100 carcases were assessed for fractures and another 100 carcases for bruising (adapted from LUDTKE et al., 2010). Scratches were scored from 0 (absence) to 3 (severe; SOUZA et al., 2018a) in 100 additional carcases, totalling 300 carcasses per flock.

In addition, data provided by the slaughterhouse regarding dead on arrival (DOA), total and partial carcase condemnations due to diseases such as ascites, arthritis, dermatosis, myopathy, airsacculitis and abnormalities. These would be rejected by Brazilian official inspectors due to colour, excreta, and sexual or abnormal odours (MAPA, 1998).

3.2.3 Environmental measurements

Environmental parameters were collected to characterise the indoor living conditions in all units simultaneously to the assessment of health indicators (TABLE 7). Data were obtained from 30 equidistant locations at bird level. Relative humidity, temperature and carbon dioxide (CO_2) were assessed with an Akso AZ 77535, as well as the external temperatures at the beginning and end of data collection. Air velocity and illuminance were measured with a Lutron LM 8000A and ammonia (NH_3) concentration by SP2nd Portable Single-Gas Detector.

TABLE 7 - MEDIAN (MIN-MAX) VALUES FOR INDOOR RELATIVE HUMIDITY, TEMPERATURE, AIR VELOCITY, ILLUMINANCE, AMMONIA (NH_3) AND CARBON DIOXIDE (CO_2) IN 10 CLOSED-SIDED AND 10 OPEN-SIDED POULTRY HOUSES, FROM JULY TO AUGUST 2019, IN THE WEST OF SANTA CATARINA STATE, SOUTH OF BRAZIL.

Variable	Poultry house		
	Closed-sided	Open-sided	P
Relative humidity (%)	74.5 (50.7–99.9)	72.3 (47.4-99.9)	0.0005
Temperature (ºC)	23.9 (14.6-29.2)	21.7 (12.9-30.1)	<0.0001
Air velocity (m s^{-1})	2.3 (0.0-7.8)	0.0 (0.0-4.3)	<0.0001
Illuminance (lx)	16.0 (1.0-60.0)	161.0 (8.0-2380.0)	<0.0001
NH_3 (ppm)	9.0 (0.0-64.0)	12.0 (0.0-60.0)	<0.0001
CO_2 (ppm)	1749.0 (864.0-5869.0)	1716.0 (1044.0-5900.0)	0.7113

For the litter moisture analysis, around 400 g was collected at 12 locations per house, avoiding areas near or below the feeders or drinkers. These samples were packed in plastic bags, identified and sent for laboratorial analysis. Following Tedesco et al. (1995), 20 to 30 g of litter samples were homogenised and placed in a forced ventilation oven for up to 48 h, or until no change in weight was observed with increasing drying time, at 65-70°C. The litter moisture averages were 34.6±7.4% for CS and 41.0±14.5% for OS houses, but they were not significantly different (P=0.618).

3.2.4 Bird behaviour and affective states

Bird behaviour was recorded with two Canon Vixia HF R800 video cameras. Two physical structures, measuring 1.5 x 1.5 m and made with steel cable, were used to mark the bird observation area on the floor. One structure was placed in the middle of the house, and the other near the wall. The behaviour of completely visible birds and of those with more than half of their bodies within the physical structure was assessed, according to a predefined ethogram (TABLE 8). Observations were made for 4 h per day, simultaneously, for each site of the house, totalling 8 h per unit. Each behaviour expressed by each bird was counted and analysed using scan sampling with instantaneous recording every 10 min (MARTIN; BATESON, 1993), during 48 time points spread over 8 h of recording. Feeding behaviour was not assessed in the observation areas next to the wall in either CS or OS houses, due to the absence of feeders within the physical structure.

TABLE 8 - ETHOGRAM USED TO RECORD BROILER CHICKEN BEHAVIOUR IN 10 CLOSED-SIDED AND 10 OPEN-SIDED POULTRY HOUSES, FROM JULY TO AUGUST 2019, IN THE WEST OF SANTA CATARINA STATE, SOUTH OF BRAZIL.

Behaviour	Definition
Feeding	Having the head in the feeder, or pecking at the feed in the feeder
Drinking	Having the beak in touch with the drinker
Foraging	Pecking and/or scratching on the floor
Exploration	Interacting with physical structures used to delimit the bird observation area
Comfort	Preening, wing flapping, wing stretching, feather ruffling or shaking, and elements of dustbathing behaviour
Inactive	Sitting, lying, or standing while not engaged in other activities, eyes are opened or closed
Locomotion	Running, walking, or jumping
Other	Any additional behaviour performed by broiler chicken other than those included in the ethogram, such as vigilance and panting. Elements of aggressive or behavioural problem towards another bird, such as threatening, leaping, kicking, wing flapping or severe feather pecking, being disturbed by another bird or disturbing another bird and positive social behaviour such as allow grooming

For the assessment of human-animal relationships, a touch test was used, in which the observer attempted to touch birds during 21 interaction sessions in each barn, recording the number of birds within an arm's length and the number of birds actually touched at each session. If no animal was within an arm's length for the first 12 attempts, the session was ended (WELFARE QUALITY®, 2009). The data was expressed as a numeric score ranging from zero to 100, with zero meaning that no animal was touched and 100 that all animals within reach were touched, based on calculations from the section good human-animal relationship of the Welfare Quality® protocol (WELFARE QUALITY®, 2009).

The Qualitative Behaviour Assessment (QBA) was performed before starting other indoor evaluation procedures. After an observation period of 10 min, the assessor recorded 25 emotional descriptors on a visual analogue scale that ranged from 0 mm, indicating that the emotion was entirely absent in the group of animals observed, to 125 mm, meaning the emotion was dominant (WELFARE QUALITY®, 2009; SOUZA et al., 2021). The terms used were the Brazilian Portuguese equivalents for scared, inquisitive, painful, relaxed, aggressive, positively occupied, lethargic, comfortable, fearful, active, dull, confident, agitated, interested, apathetic, playful, desperate, apprehensive, attentive, distressed, calm, frustrated, lively, disturbed and tranquil responses, developed for Brazilian Portuguese native speakers (SOUZA et al., 2021).

3.2.5 Statistical analysis

Differences in stocking density, house size, flock size, age at the time of the visit, age and body weight at slaughter, mortality, culls, touch test and litter moisture were analysed by a t-test for two independent samples.

For contact dermatitis on the breast and abdominal areas, bird soiling, footpad dermatitis, hock burn and lameness, the house effect was assessed using a proportional odds regression model. The results were presented as estimated odds ratios. Due to the low frequencies of results, scores for some indicators were aggregated as follows; contact dermatitis on the breast and abdominal areas, where condition (C) 1 corresponded to the 0 score, C2=1 and C3=2+3; for bird soiling, C1=0, C2=1 and C3=2+3; for footpad dermatitis, C1=0, C2=1 and C3=2+3+4; for hock burn, C1=0, C2=1 and C3=2+3+4; and for lameness, C1=0+1+2 and C2=3+4+5. For lameness, a usual binary logistic regression model was fitted. For other variables, the proportional odds regression model was used and assessed by the likelihood ratio test. If rejected, as an alternative the fit of separate regression models for each variable was performed. In all cases, the house effect was included as a random variable with a normal distribution of means equal to constant variance σ^2.

Data from the slaughterhouse were analysed using generalised linear models. The half normal plot for residuals with simulated bands was used in order to detect overdispersion or any other source causing lack of fit. For fractures, bruises and scratches, a binary logistic regression model was used, and for scratches, a proportional odds regression model was used. For DOA and diseases, a regression model with a Poisson response was initially fitted; however, due to data overdispersion, a negative binomial regression model was used. The negative binomial distribution allowed for the incorporation of the additional variation present in the data which had not been accounted for by the type of house, *i.e.,* factors specific to housing. At this stage, the only explanatory variable considered was the type of house and the (log) number of animals in each.

Normality of the data distribution was determined by the Shapiro Wilk test for external and internal environmental measurements, such as relative humidity, temperature, air velocity, illuminance, NH_3 and CO_2 concentrations. Statistics were calculated and compared using the Mann-Whitney test.

The data from bird behaviour assessment were analysed by fitting regression models appropriate for counted data. As response variable, the registered frequencies of the animals for each behaviour were considered, resulting in a single frequency *per* house type, and, as explanatory variables for types of behaviour, the type of housing and measurement location, middle or wall were included. A possible effect of access to feeders and drinkers were adjusted for each behaviour. Furthermore, the (log) total frequencies of animals in each barn was included as a covariate in this model. The use of log-linear regression models with negative binomial responses allowed the accommodation of the overdispersion present in the data. In cases of multiple comparisons, the P-values were adjusted using the Tukey method in order to maintain the global level of significance at 5%.

Principal component analysis (PCA) was conducted, in order to exploit the correlation of the 25 investigated features for QBA. The number of components retained for analysis was determined through simulation, using parallel analysis. Four components explained most of the variance in the data for each type of house. The PCA scores were compared by t-test. All analyses were performed based on a significance level of 5%, using R software (R CORE TEAM, 2019), through the ordinal and lme4 package (BATES et al*.,* 2015).

3.2.6 Ethical approval

This project was approved by the Animal Use Ethics Committee of the Agricultural Campus (No 046/2018), of the Federal University of Paraná (ANNEX I, II).

3.3 RESULTS

From the 32 statistical comparisons regarding welfare indicators, 12 showed significant differences between housing types. Six of these 12 differences were more positive in CS houses, with two indicators related to environmental conditions, namely air velocity and NH_3, and four being animal-based, namely contact dermatitis on the breast and abdominal areas, bird soiling, hock burn and touch test. The other six differences indicated better conditions in OS houses, three related to environmental conditions (*viz*. relative humidity, temperature and illuminance) and two being animal-based (*viz*. drinking and inactive behaviours). The QBA results were better for OS houses. Overall, the results suggested that bird welfare was low in general, with each type of house limiting the welfare of animals in various aspects.

3.3.1 Health assessments

There were differences ($P<0.05$) between CS and OS houses for three health indicators assessed on farm, including contact dermatitis on the breast and abdominal areas, hock burn, and bird soiling (TABLE 9), and their percentages are shown in FIGURE 5. Overall, birds reared in CS showed less chances of severe lesions than OS houses.

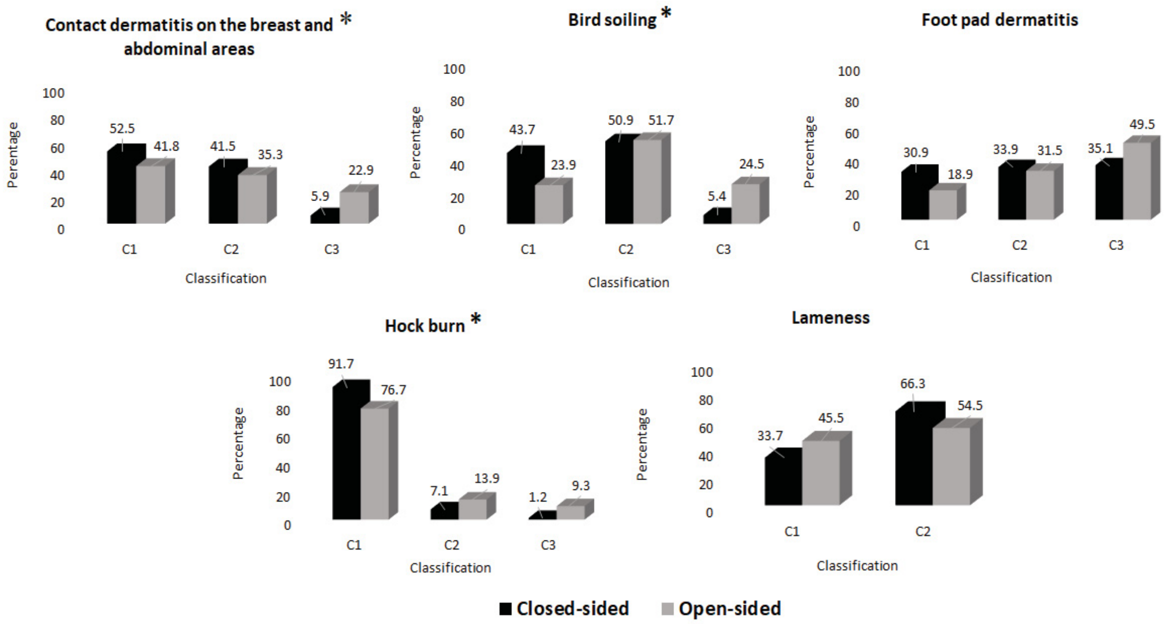
TABLE 9 - ESTIMATED ODDS RATIOS FOR WORSE SCORES ON CONTACT DERMATITIS ON THE BREAST AND ABDOMINAL AREAS, BIRD SOILING, FOOTPAD DERMATITIS, HOCK BURN AND LAMENESS FOR 10 CLOSED-SIDED RELATIVE TO 10 OPEN-SIDED POULTRY HOUSES, FROM JULY TO AUGUST 2019, IN THE WEST OF SANTA CATARINA, SOUTH OF BRAZIL.

Variables	Closed- / Open-sided poultry houses			
	Scores	Odds ratio	CI (95%)	P
Contact dermatitis on the breast/abdominal areas	1 / 0	0.71	(0.33; 1.49)	0.367
	2+3 / 0	0.19	(0.04; 0.93)	0.040
	2+3 / 1	0.29	(0.12; 0.71)	0.007
Bird soiling	1 / 0	0.37	(0.15; 0.91)	0.031
	2+3 / 0	0.07	(0.00; 0.63)	0.016
	2+3 / 1	0.22	(0.05; 0.92)	0.038

Footpad dermatitis	- [1]	0.42	(0.13; 1.36)	0.150
Hock burn	1 / 0	0.38	(0.12; 1.14)	0.086
	2+3+4 / 0	0.14	(0.03; 0.71)	0.017
	2+3+4 / 1	0.41	(0.16; 1.03)	0.059
Lameness	0+1+2 / 3+4+5	1.78	(0.88; 3.61)	0.106

[1] "-" The assumption of proportional reasons for footpad dermatitis was verified to allow for the estimation of a single odds ratio.

FIGURE 5 - OVERALL MEAN PERCENTAGE OF CONTACT DERMATITIS ON THE BREAST AND ABDOMINAL AREAS (C1=0 SCORE, C2=1 AND C3=2+3); BIRD SOILING (C1=0, C2=1 AND C3=2+3); FOOTPAD DERMATITIS (C1=0, C2=1 AND C3=2+3+4); HOCK BURN (C1=0, C2=1 AND C3=2+3+4); AND LAMENESS, (C1=0+1+2 and C2=3+4+5); * DENOTES A SIGNIFICANT DIFFERENCE.



There were no statistical differences for slaughterhouse data. The average DOA was 0.12% ± 0.08 in CS and 0.09% ± 0.06 birds in OS houses (P=0.374). The means for disease prevalence in CS and OS houses were 0.15% ± 0.08 and 0.09% ± 0.05 for ascites (P=0.061), 0.30% ± 0.17 and 0.22% ± 0.10 for arthritis (P=0.060), 0.94% ± 0.74 and 0.34% ± 0.21 for dermatosis (P=0.103), 1.93% ± 0.91, 1.89% ± 0.58 for myopathy (P=0.986), 0.06 ± 0.04, 0.05% ± 0.02 for airsacculitis (P=0.113), and 0.11% ± 0.08 and 0.07% ± 0.04 for abnormalities (P=0.365), respectively. The odds ratio for scratches in birds housed in CS in relation to OS houses was 0.71:1 (P=0.379). Scratch prevalence in CS housing compared to OS barns were 23.4% and 22.0% (0 score), 41.0% and 32.8% (1 score), 22.8% and 28.0% (2 score), and 12.7% and 17.2% (3 score), respectively. Bone fracture prevalence was 0.02% and 0.04% (P=0.845), and bruising 0.18% and 0.26% (P=0.240), respectively for CS compared to OS housed birds.

3.3.2 Bird behaviour and affective states

Only two behaviours presented statistical differences between housing types (TABLE 10). There were lower rates for drinking (P=0.007) and higher for inactivity (P<0.001) in CS than OS houses. The inactive behaviour accounted for 65.0% of total behavioural activities in CS and 57.2% in OS houses, drinking (8.0% and 11.1%), comfort (9.7% and 8.9%), feeding (6.6% and 8.3%), locomotion (4.4% and 5.0%), foraging (3.0% and 3.8%), other (2.3% and 4.1%), and exploration (1.0% and 1.6%), respectively.

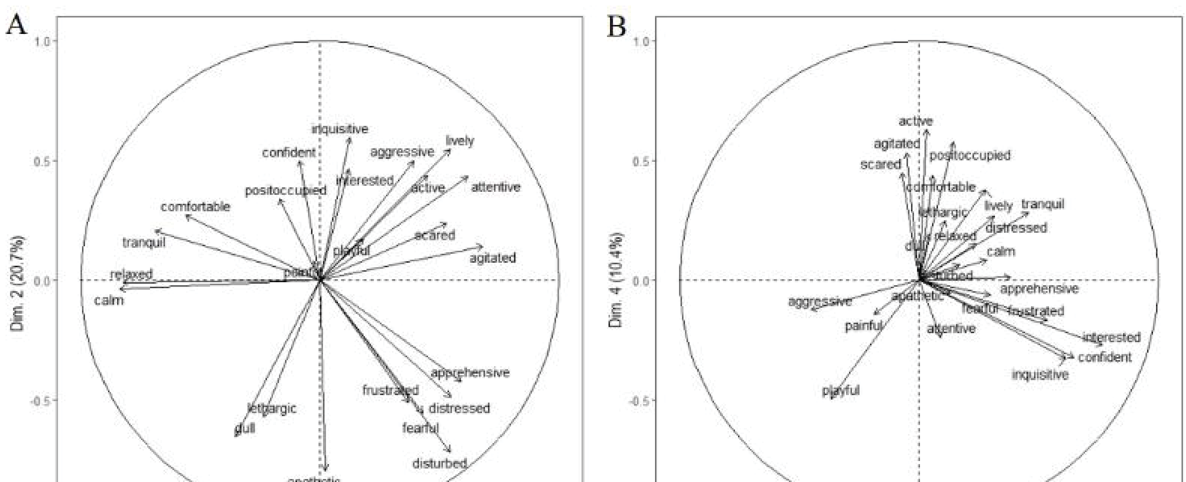
TABLE 10 - RELATIVE RATES OF BEHAVIOURS FOR 10 CLOSED-SIDED RELATIVE TO 10 OPEN-SIDED POULTRY HOUSES, FROM JULY TO AUGUST 2019, IN THE WEST OF SANTA CATARINA STATE, SOUTH OF BRAZIL.

Behaviour	Relative rate	CI (95%)	P
Feeding	0.76	(0.55; 1.02)	0.075
Drinking	0.70	(0.53; 0.90)	0.007
Foraging	0.79	(0.59; 1.03)	0.089
Exploration	0.66	(0.40; 1.09)	0.110
Comfort	1.10	(0.95; 1.25)	0.175
Inactive	1.14	(1.05; 1.23)	<0.001
Locomotion	0.88	(0.73; 1.04)	0.153
Other	0.51	(0.24; 1.06)	0.073

The touch test scores (min-max) were 98 (96-100) in CS and 67 (25-100) for birds in OS houses (P<0.001). The mean number of birds within arm's reach per attempt was 2.1±2.0 birds in CS and 1.3±1.3 birds in OS houses. The number of broiler chickens actually touched was 1.5±1.6 and 0.7±0.9, respectively. In one farm OS housing, no birds were touched after 12 trials, and, consequently, the session was abandoned.

Principal component analysis of the 25 QBA terms revealed four principal components, which explained, 23.0%, 20.7%, 12.1% and 10.4% of the total variance, totalling 66.2% of the original variance (FIGURE 6). The first component average scores and standard deviations for CS and OS houses, were -0.54±2.36 and 0.54±2.47 (P=0.334), second component -1.10±2.28 and 1.10±1.80 (P=0.028), third component 0.01±1.52 and -0.01±2.04 (P=0.976), and fourth component -0.23±1.19 and 0.23±2.00 (P=0.545). There was only statistical difference between CS and OS houses for the second component, which suggested a bird disposition dimension. Open-sides houses had higher incidence, for the most part, representing positive emotions, which ranged from inquisitive, confident, aggressive, interested, lively and positively occupied, to apathetic, dull, lethargic, disturbed, fearful and distressed, representing negative emotions with higher incidence in CS houses. As for the components that did not differ between house types, the first component presented emotional states ranging from agitated, apprehensive, scared and distressed, to calm, relaxed, tranquil, and comfortable. The third component ranged from interested, confident, inquisitive and frustrated, to aggressive, painful, and playful; and the fourth component from active, agitated, positively occupied, comfortable, playful, inquisitive, confident, interested and frustrated.

FIGURE 6 - PRINCIPAL COMPONENTS 1 AND 2 (A), 3 AND 4 (B) LOADINGS FOR EACH QUALITATIVE BEHAVIOUR ASSESSMENT TERMS ACROSS THE FOUR PRINCIPAL COMPONENTS, FOR 10 CLOSED-SIDED AND 10 OPEN-SIDED POULTRY HOUSES, FROM JULY TO AUGUST 2019, IN THE WEST OF SANTA CATARINA STATE, SOUTH OF BRAZIL.



3.4 DISCUSSION

During the winter season, birds reared in OS houses were more likely to have more severe scores for contact dermatitis on the breast and abdominal areas, hock burn and bird soiling as compared to those reared in CS houses (TABLE 9). According to Part et al. (2016), during wintertime in a temperate climate, the welfare of a large number of broiler chickens, when assessed at slaughter, was compromised, showing higher prevalence of ascites. This problem may become more intense in winter due to inadequate thermal control, such as decreased air renewal, that may, in turn, lead to increased relative humidity and litter moisture. This has been related to increased food intake and metabolic oxygen requirement, leading to pulmonary hypertension (JULIAN, 1993; CORDEIRO et al., 2010; PART et al., 2016).

For tropical climates, there are other types of health problems that may decrease bird welfare. Hock burn, breast blisters and footpad dermatitis may be summarised under the expression ‘contact dermatitis’ (MELUZZI; SIRRI, 2009). Footpad dermatitis is one of the main lesions observed during intensive chicken rearing; however, it is possible that this outcome be associated with inadequate husbandry regarding litter moisture and higher NH_3 concentrations. These environmental factors may be exacerbated by the weight of the birds and stocking density, which compromises animal welfare and increases prevalence of other injuries, such as breast blisters and hock burn (MELUZZI; SIRRI, 2009; ALLAIN et al., 2009; DE JONG et al., 2012; SARAIVA; SARAIVA; STILWELL, 2016). Sans et al. (2021) observed more contact dermatitis on the breast and abdominal areas in birds from CS compared to OS houses during the summer and autumn in the same Brazilian region. This suggested that some results, such as less NH_3 and CO_2 concentrations in OS houses which are season-dependent, may improve the indoor environment, and consequently, broiler chicken welfare. On the other hand, although there were no statistical differences between house designs for footpad dermatitis prevalence, there was high prevalence of scores 2+3+4 within both house types; 35.1% in CS and 49.5% in OS houses. This tended to have decreased prevalence for severe scores in the same season. This information reinforced that contact dermatitis, especially on the footpad, is an animal welfare problem that is common and recurs in intensive poultry systems, especially during the winter, a season with higher prevalence than summer and autumn (SHEPHERD; FAIRCHILD, 2010; SANS et al., 2021). As stated previously, during the winter, litter quality is compromised due to reduced ventilation rates and increased relative humidity, which, in turn, may lead to increased skin injuries (MELUZZI et al., 2008).

In the current study, results for litter moisture and reused litter showed no differences according to house type. In general, litter reuse deserves careful consideration, as keeping the litter dry until the end of the production cycle becomes a great challenge. Accumulation of waste and the lack of adequate management tends to lead to the generation of gases (including NH_3) from the microbial decomposition in the litter (SAKAMOTO; BENINCASA; SILVA, 2020). Despite indoor climate control systems, seasonal patterns were observed in this study and other studies (SHEPHERD; FAIRCHILD, 2010; SANS et al., 2021), which suggested that indoor systems might not provide as much protection from weather conditions as it may be initially thought. Part et al. (2016) reported the influences of weather on the indoor environment of intensive systems. The importance of considering the climatic conditions of each region for improving broiler chicken welfare when planning barns has been recognised before (ABREU; ABREU, 2011). Thus, both climatised and semi-climatised barns offer some protection to external climatic conditions but are not impermeable to them.

Controlling temperature, relative humidity and ventilation is crucial, and these may directly influence important aspects of broiler chicken welfare (JONES; DONNELLY; DAWKINS, 2005; NÄÄS et al., 2014). The temperature, relative humidity, air velocity and stocking density were higher in CS than OS houses. Although the first two indicators were different between barn types, both showed inadequate values (TABLE 7), with temperature exceeding recommendation of 20ºC for six-week-old chickens (ABREU; ABREU, 2011), and 70% for an acceptable relative humidity (ABREU; ABREU, 2011; ROSS, 2014; COBB, 2018). The same situation was observed during summer and autumn for the same region, with values above those recommended in both house types (SANS et al*.,* 2021). Thus, the main conclusion from these parameters was that the situation is currently inadequate in both types of barn throughout the year and may be worse in summer season.

Air velocity was within recommended parameters of 1.7-3.0 m s^{-1} for birds over 28 d of age (COBB, 2012). It is known that high stocking density negatively influences many factors related to chicken welfare, for example, by increasing the litter moisture, which then affects walking ability and enhances the risk for contact dermatitis and thermal discomfort (SAKAMOTO; BENINCASA; SILVA, 2020). During the winter, the current results suggested that birds may suffer from thermal stress; however, this situation may be worse during summer and autumn, as seen by elevated levels of panting (SANS et al., 2021). It thus emphasises the importance of controlling internal environmental conditions, in both house types and in all seasons. A factor that may have alleviated the poor internal environmental conditions was air velocity, especially in CS houses where it was higher during summer and autumn seasons (SANS et al., 2021). The temperature and relative humidity, altogether with proper ventilation, may provide adequate thermal sensations for birds. Good ventilation also promotes greater air renewal (NÄÄS et al., 2014; PAULINO et al., 2019), which in turn lowers gas concentration (DE JONG et al., 2012). This means that animal welfare may not depend on just one environmental parameter within recommended levels, but all parameters require daily monitoring and corrections, according to animal behaviour and physiological needs.

Although NH_3 and CO_2 concentrations, on average, did not exceed the respective thresholds of 20 and 3000 ppm (DE JONG et al., 2012; ROSS, 2014) in any of the house types studied; NH_3 was significantly higher in OS barns, most likely related to the lower automatic ventilation rates. Gases may reach toxic levels, which cause health risks to both birds and workers (PAULINO et al*.,* 2019). For CO_2, a level of 1% does not, by itself, cause any harm for animals. However, higher CO_2 concentration are usually accompanied by increased levels of other detrimental air pollutants, such as NH_3, dust and micro-organisms. Therefore, CO_2 may be considered a relevant indicator of air quality (DE JONG et al., 2012). The most known air pollutant in poultry houses is NH_3, and it can be harmful when in contact with feet and leg skin (NASEEM; KING, 2018; SOUSA et al., 2018). Higher NH_3 may provide explanation for the higher prevalence of dermatitis in birds reared in OS houses, as humid and hot litter releases more NH_3 (MARTINS; HÖTZEL; POLETTO, 2013). In general, gas concentrations are lower in open-sided houses, due to the handling of the curtains favouring air renewal (NÄÄS, 2008). Nonetheless, during the winter, a higher concentration of gases is common (PAULINO et al., 2019). In the current study, the adjustment of curtain sides was relatively infrequent for avoiding heat loss from the house. This appeared to be a critical result for OS houses and was related to lower bird welfare in this type of barn during the winter season. During summer and autumn, lower NH_3 and CO_2 concentrations have been observed for OS houses, due to frequent adjustment of the curtain sides (SANS et al., 2021). These results supported the recognition of the important role of ventilation to maintain good air quality (DE JONG et al., 2012).

The higher prevalence of bird soiling in OS houses suggested poorer environmental conditions. Litter quality is an important risk factor for bird welfare, since chickens spend all their lives kept on litter material (SARAIVA; SARAIVA; STILWELL, 2016; ÇAVUŞOĞLU; PETEK, 2019). Accordingly, some studies have added to this information by demonstrating a positive correlation between litter quality and plumage cleanliness (FEDERICI et al., 2016; SARAIVA; SARAIVA; STILWELL, 2016). Although the current trial showed increased feather soiling in birds from OS houses, Tuyttens et al. (2015) observed that chicken flocks had cleaner plumage when raised in OS houses in Brazil during the spring season compared to CS houses in Belgium. Such controversial results reinforced the importance of monitoring this indicator, regardless of poultry house type, as other factors seem to interact with the relationship between environment and bird cleanliness, such as season (SANS et al., 2021) and geographical location, which in turn may aggravate differences in management.

Different illuminance values were observed inside the barn for both house types, with median results of 16.0 lx (1.0-60.0 lx) in CS houses, which was below the minimum illuminance of 20 lx recommended for broiler chicken welfare (DE JONG et al., 2012). In the OS houses, the illuminance median was 161 lx (8.0-2380.0 lx). Lighting was worse during summer and autumn, with a mean of 6.9±6.3 lx in CS houses (SANS et al., 2021), and such differences is likely related to management decisions between seasons. Illuminance, as well as the type of lamps used in the poultry industry, may influence welfare. Under 1 lx, basic eye functional characteristics, such as eye size, are impaired (DEEP et al., 2010) and the behavioural repertoire may decrease in birds reared at an illuminance of 5 lx when compared to 20 lx (RAULT et al., 2016). Low lighting may induce birds to remain in a constant apathetic state (PARANHOS DA COSTA; LIMA; SANT'ANNA, 2017). Additionally, the lack of standardisation of lamp types used, such as incandescent and fluorescent lights, has been related to limitations in providing suitable levels of ultraviolet, which is important for mediated behaviours, such as fear and stress responses (SOBOTIK; NELSON; ARCHER, 2019). According to James et al. (2018), chickens subjected to UVA and UVB light showed improved feathering, lower fearfulness and better walking ability, which suggested that their incorporation to commercial poultry houses may be beneficial for bird welfare. Despite these results, maintaining birds in indoor conditions under low illuminance is the poultry industry standard. This has been complicated by recommendations from breeder companies advising the provision of indoor illuminances of around 5-10 lx (ROSS, 2014; COBB, 2018). Recently, the Brazilian poultry industry has shown a trend in changing from OS to CS houses (SOUZA; MOLENTO, 2015), with a likely decrease in illuminance. However, as bird welfare problems may be alleviated with adequate levels of UV exposure through natural light provision in OS houses or by LED lighting systems that include UV, this is an area where more research is needed.

Overall, the results for bird health were less positive for animals reared in OS houses during the winter time. For environmental indicators, relative humidity and temperature showed statistical differences, with better results in OS houses; however, both values were above recommendations. Lower NH_3 concentration was observed in CS houses, but within recommended levels in both house types. Illuminance was better in OS houses, being above recommendations, while for CS houses were outside normal animal welfare requirements (EFSA, 2012). In general, welfare was compromised in both house types, apparently being worse regarding health indicators in OS houses and environmental conditions in CS houses. The inclusion of other analyses for litter quality, such as pH and temperature, as well as compaction, appear to be important to generate additional relevant information for better understanding of health and environmental conditions faced by birds in each house type.

Differences were observed for bird behaviour, with higher frequency for inactivity and lower drinking behaviour in CS houses (TABLE 10). Many welfare problems arise when animals cannot perform their natural behaviour, and it has been recognised that current intensive production systems are unable to meet the natural needs of the birds (EL-DEEK; EL-SABROUT, 2019; SANCHÉZ-CASANOVA et al., 2020). Sans et al. (2021) observed more exploratory and panting behaviour in OS than CS houses during the summer and autumn in the same region in Brazil, which demonstrated difficulties in meeting bird behavioural needs in both barn types. The highly modified genetics of commercial strains has led chickens to spend from 76% to 86% of their time inactive, varying according to age and any locomotor problems (WEEKS et al., 2000). The high level of inactivity is an important welfare problem, which may relate to other welfare problems, such as contact dermatitis and leg abnormalities (BESSEI, 2006). The lower illuminance in CS houses may contribute to decreased behavioural expression, such as more inactivity, as birds increase time spent in other activities if stimulated with natural lighting and environmental enrichment (BAILIE; BALL; O'CONNELL, 2013; BACH et al., 2019). De Jong, Gunnink (2018) reported that providing enrichment alone may not be efficient to stimulate activity in broiler chickens, and, to get a real increase in natural behavioural, it seems important that natural light is provided simultaneously. Sans et al. (2021) observed more exploratory behaviour during summer and autumn in OS compared to CS houses, which suggested that natural light encourage an increase in the bird’s behavioural repertoire, especially exploratory behaviour. Thus, to increase bird welfare status, it is important that chickens have more possibilities for interacting with available house environment, since behavioural limitations are prevalent in the industrial system, regardless of season or house type.

In relation to drinking behaviour, the results were contradictory, as birds reared in CS houses had less access to the drinkers, even though the mean temperature was higher when compared to OS houses (TABLE 7). It has been reported that birds kept under high temperatures tend to increase water intake (SAEED et al., 2019). Hence, characteristics other than temperature contributed to the increased drinking behaviour seen in OS barns, and the more active behaviour in this house type may be potentially related. Bailie, Ijichi, O’Connell (2018) did not observe differences in lying down behaviour for birds reared in windowed houses; however, they suggested a decrease in water consumption per 1000 birds as stocking density increased. The same authors confirmed that broiler chickens were more active and used enrichments, such as perches and string, when they were provided, along with exposure to 62.9 and 63.9 lx (natural light). Birds reared under low illuminance are typically less active, which may be related to the lower occurrence of drinking. Light is important in the regulation and control of bird behaviour and health, and its restriction can lead to changes in behaviour (SANCHÉZ-CASANOVA et al., 2020). This rationale warrants further study, including water quality, due to its potential to influence intake (CITADIN, 2014). However, a static environment, with low illuminance and higher rates of inactive behaviour, seems to be related with the lower water intake observed.

The touch test showed differences in scores (min-max) of 98 (96-100) for CS and 67 (25-100) for OS houses (P<0.001). According to Welfare Quality® (WELFARE QUALITY®, 2009), birds will withdraw from the observer if they are fearful, and the current study indicated higher avoidance reactions for birds in the OS houses. Regular and positive physical contact may reduce fear and avoidance from broiler chickens to humans, and thus improve welfare (JONES, 1993; HEMSWORTH, 2003). However, creating space and providing an enriched environment may be an effective method of stimulating certain bird behaviours and decreasing others, such as fear reactions, when compared to sterile environments (BAXTER; BAILIE; O'CONNELL, 2018). Natural lighting and environments with higher illuminance appear to play an important role in increasing bird activity (BESSEI, 2006; BAILIE; BALL; O'CONNELL, 2013). Thus, the touch test results may differ for reasons other than increased fear (FEDERICI et al., 2016), such as with reduced walking ability (VASDAL et al., 2017). Sans et al. (2021) indicated a possible relationship between the increased numbers of touched bird with higher prevalence of severe lameness scores (3 and 4). Riber et al. (2018) considered it important to conduct more research in commercial conditions, regarding the interaction of enrichment and other factors, such as natural light, stocking density and flock size, to answer questions regarding fearfulness. It is possible that the greater intensity of light and the lower stocking density observed in OS houses provided the chickens with more chances and greater ability to avoid physical contact. Thus, it seems inadequate to interpret lower touch test scores as an indicator of fearfulness and lower welfare state. It is possible that the touch test is not useful to compare differences in fearfulness between systems that differ in many other aspects relevant for bird movement, such as illuminance, inactive behaviour and stocking density.

Results from the QBA showed statistical differences in emotional states. The second component indicated that birds reared in OS houses showed higher prevalence of positive emotional states, such as active, confident, interested, attentive and lively, and less related to negative emotional states, including lethargic, fearful, dull, apathetic, apprehensive, distressed, frustrated and disturbed behaviour. Animals can express pleasure in various activities, such as resting, dust bathing, eating, running, social interaction, comfort, hygiene and enjoying the sun, as well as express unpleasant feelings in situations such as hunger, thirst, pain and frustration (APPLEBY; MENCH; HUGHES, 2004; KUMAR et al., 2019). As such, birds reared in the OS houses likely had slightly greater chances of meeting more of their basics needs, resulting in less pronounced negative emotional states. Animal welfare is primarily related to the feelings experienced by animals, and high welfare states refer to the absence of negative feelings known as ‘states of suffering’ and to the presence of positive feelings known as ‘states of pleasure’ (DUNCAN, 2005). Association between QBA and the touch test has been discussed by Muri et al. (2019), who reported that birds with greater liveliness that had less acceptance for being touched, and, consequentially, were less likely to have higher mortality. According to Boissy et al. (2007), the absence of positive emotional states in animals is, in itself, sufficient to qualify as an affective state of discomfort. Results obtained in the present study suggested that, even though some welfare indicators were worse in OS houses, these undesirable conditions were not sufficient to result in less positive emotional states as compared to CS houses. However, this did not alleviate the need for improvements in OS houses. Although the OS houses seemed related to relatively lower behavioural restrictions and more positive emotional states, in both types of housing some of the birds’ behavioural needs were lacking. These results strengthen the argument put forward by Relić et al. (2019), who suggested incompatibility between intensive poultry production and natural behaviour. In general, there are differences in bird welfare, which may vary according to the season and indoor conditions for each house type. Farmers may assist by monitoring welfare and adjusting the environmental conditions as needed, providing a more comfortable environment for broiler chickens. However, the current results showed the important limitations that industrial intensive poultry systems impose on animal welfare, regardless of season and barn type.

3.5 CONCLUSIONS

For the first time, it has been shown that critical restrictions for broiler chicken welfare in different types of barn vary according to season. During winter, in contrast to previously published results for summer and autumn, CS poultry houses showed better results for contact dermatitis on the breast and abdominal areas, bird soiling, hock burns, air velocity, NH_3 concentration and touch test, compared to OS barns. Results for stocking density, relative humidity, temperature, illuminance, inactivity, drinking behaviour and QBA were more positive in OS houses. Thus, in winter, CS houses have fewer welfare problems in terms of health indicators, while OS houses have fewer behavioural restrictions and more positive emotional states. Overall, bird welfare in both types of housing was compromised by many restrictions, such as high flock density, contact dermatitis, environmental indoor conditions, a barren environment and low behavioural repertoire, which calls for attention to house-specific as well as common welfare problems

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4. IN-BARN HETEROGENEITY OF BROILER CHICKEN WELFARE IN TWO INDUSTRIAL HOUSE DESIGNS AND TWO SEASONS IN SOUTHERN BRAZILIAN SUBTROPICAL CLIMATE

RESUMO

A ciência do bem-estar animal é fundamental para melhorar a qualidade de vida de bilhões de aves, apoiando decisões por meio da avaliação de indicadores ambientais e baseado nos animais em diferentes condições de criação. Nosso objetivo foi avaliar a variação do bem-estar dos frangos de corte dentro do mesmo tipo de galpão e se esta variação era dependente do tipo de instalação e da estação do ano. Neste estudo, descrevemos e comparamos a heterogeneidade interna do bem-estar das aves em dois diferentes tipos de galpão industriais, sendo quatro galpões do tipo dark-house (CS) e 13 semi-climatizados (OS), durante duas diferentes estações do ano (verão/outono e inverno). As avaliações foram divididas em duas categorias: 1) indicadores ambientais: umidade relativa, temperatura, velocidade do ar, concentrações de amônia (NH_3) e dióxido de carbono (CO_2) e iluminância; 2) indicadores baseados nos animais: dermatites de contato na região do peito e abdômen, limpeza das aves, pododermatite, queimadura de jarrete e claudicação. Os resultados das avaliações que cobriram 30 locais equidistantes dentro de cada tipo de galpão, foram organizados em mapas de krigagem. Foram efetuados ajustes de regressão linear e modelos generalizados, considerando variáveis preditoras e o efeito de interação entre os mesmos; o teste Tukey foi usado para as comparações múltiplas das médias. Modelagem geoestatística foi utilizada para dados contínuos e discretos, para os dados referentes aos indicadores ambientais e baseados nos animais, respectivamente. Foi observada heterogeneidade dentro dos galpões para a prevalência de problemas ambientais e baseados nos animais. Houve um padrão para a distribuição espacial, na direção do centro para a extremidade Oeste, em ambos os tipos de instalações. Os piores resultados foram observados para três indicadores ambientais (temperatura, concentrações de NH_3 e CO_2) e três indicadores baseados nos animais (queimadura de jarrete, limpeza das aves e pododermatite). Em galpões CS, a iluminância foi muito restritiva (de 4,4 a 6,7 lx) quando comparada à OS (de 119,8 a 145,3 lx); em ambos os tipos de galpão, a prevalência de claudicação foi alta (de 50,9 a 78,0%), embora as prevalências de iluminância e claudicação estivessem uniformemente distribuídas em ambos os tipos de galpão. Os mapas de krigagem permitiram a identificação de piores problemas de bem-estar na direção Oeste, local o qual nos galpões CS significa próximo aos exaustores e em OS, na direção da ventilação mecânica de pressão positiva efetuada pelos ventiladores. Os resultados mostram que é necessária atenção para a variação das condições de bem-estar das aves dentro de cada tipo de galpão, e permitem a adoção de estratégias para disseminar as melhores condições para a área interna em ambos os designs de instalação. Principalmente, as descobertas originais sobre a heterogeneidade do bem-estar dos frangos de corte sugerem a relevância do monitoramento constante em locais-chave dentro de cada galpão, no mínimo para os indicadores com diferentes distribuições espaciais dentro de cada instalação.

Palavras-chave: ambiente, aves, geoestatística, indicador baseado nos animais, mapas de krigagem.

ABSTRACT

The science of animal welfare is key to improving the life quality of billions of chickens, by supporting decisions through the assessment of environmental and animal-based indicators in different housing conditions. Our goal was to assess the variation of bird welfare within the same barn and whether this variation depends on barn type or season. We described and compared the in-barn heterogeneity of broiler chicken welfare in four closed-sided (CS) and 13 open-sided (OS) industrial poultry houses, during two different seasons (summer/autumn and winter). The measures were divided into two categories: 1) environmental indicators: relative humidity, temperature, air velocity, ammonia (NH_3) and carbon dioxide (CO_2) concentrations, and illuminance; 2) animal-based indicators: contact dermatitis on the breast and abdominal areas, bird soiling, footpad dermatitis, hock burn, and lameness. The results of assessments in 30 equidistant locations, covering the whole inside area of each barn, were organized into kriging maps. Linear regression and generalized models were fitted, considering predictor variables and the interaction effect between them; the Tukey test was used for the multiple comparisons of means. We used geostatistical modelling for continuous and discrete data for environmental and animal-based measurements, respectively. In-barn heterogeneity was observed for the prevalence of environmental and animal-based problems. There was a pattern for the spatial distribution, heading from the house centre to the West end of both house types. Worse results were observed for three environmental indicators (higher temperature, and NH_3 and CO_2 concentrations) and three animal-based indicators (higher prevalence of hock burn, bird soiling and footpad dermatitis). In CS, illuminance was very restrictive (4.4 to 6.7 lx) when compared to OS houses (119.8 to 145.3 lx); in both house types the prevalence of lameness was high (50.9 to 78.0%), even though both illuminance and lameness prevalence were evenly distributed inside all houses. The kriging maps allowed for the identification of worse welfare problems in the West direction, which in CS means near exhaust fans and in OS houses the direction of positive-pressure mechanical ventilation by fans. Our results show that attention is needed for the variation of bird welfare conditions inside each barn, and allow for the adoption of strategies to spread best conditions throughout the internal barn area in both house designs. Principally, the original findings on in-barn bird welfare heterogeneity suggest the relevance of constant bird welfare monitoring in key locations within the barns, minimally for the indicators with known different in-barn spatial distributions.

Keywords: animal-based, bird, environment, geostatistics, health, kriging maps.

4.1 INTRODUCTION

The science of animal welfare is gaining increasing attention and recognition, as it contributes to the understanding of animal needs, motivations and mental lives. The growing concern regarding animal welfare by researchers, companies, and governments is significantly powered by questions raised by society regarding the way animals are cared for (EU, 2015; QUEIROZ et al., 2018; ALONSO; GONZÁLEZ–MONTAÑA; LOMILLOS, 2020).

The poultry meat industry involves a large number of broiler chickens, the largest one for terrestrial vertebrates used in food production. This fact puts high priority on chicken production systems in terms of demand for animal welfare improvements (BROMM, 2001; ROWE; DAWKINS; GEBHARDT-HENRICH, 2019), in identifying critical welfare points that guide more responsible management practices. Several factors related to housing and management are known to influence chicken welfare (LOUTON et al., 2018). For example, decisions regarding house types or equipment such as natural and artificial light, lamps types, fans, evaporative cooling systems, exhausting fans and sprinklers, which in turn influence illuminance, temperature, relative humidity, ventilation and air quality, may pose several challenges to professionals (ABREU; ABREU, 2011; LIMA; SILVA, 2019) and may compromise bird welfare, if decisions are not taken according to the needs of the animals.

In broiler chicken intensive systems in Brazil, the birds are raised in two main types of barn, semi-climatized and climatized houses (ABREU; ABREU, 2011; LIMA; SILVA, 2019; SANS et al., 2021a*,* 2021b *in press*), and the proportion of each house type in this industry is a dynamic issue, with a trend for switching from semi to fully climatized barns (SOUZA; MOLENTO, 2015). The semi-climatized house type is characterized by open-sides, natural light complemented by artificial light, adjustable curtains, positive-pressure fans and sprinklers. The climatized house is completely closed with double fixed curtains on side walls, artificial light, negative pressure and evaporative cooling systems, exhausting fans and sprinklers (ABREU; ABREU, 2011; LIMA; SILVA, 2019; SANS et al., 2021a). In both house designs, the litter tends to be reused several times, a common practice in Brazil due to its high cost or a shortage of this material in some regions (CARVALHO et al., 2011; CAMPOS et al., 2018; SAKAMOTO; BENINCASA; SILVA, 2020). In general, closed-sided houses may provide better control of internal environmental conditions, while open-sided houses tend to have better air quality (NÄÄS, 2008; LIMA et al., 2020). On the other hand, Louton et al. (2018) did not find a significant difference between closed- and open-sided barns for plumage soiling (a similar analysis to bird soiling which is more focused on the feathers), footpad dermatitis, hock burn and gait score, but observed higher NH_3 concentration and poor litter quality in open-sided houses. Sans et al. (2021a, 2021b, *in press*) also observed that many welfare indicators may vary according to the house type and their management, as well as season of the year.

The internal environmental conditions of a poultry house, such as temperature, relative humidity, litter quality, ventilation, illuminance (NÄÄS et al., 2014; RAULT et al., 2017) and gas concentrations such as ammonia (NH_3) and carbon dioxide (CO_2; PAULINO et al., 2019), influence broiler chicken welfare, especially when they fall out of the natural range for the birds, for example, the case of the thermoneutral zone for environmental temperatures (FERRAZ et al., 2020). There are regulated limits for maintaining the bird environment, such as relative humidity (45-70%), NH_3 (10-20 ppm), CO_2 (<3000 ppm) and a minimum of 20 lx of illuminance measured at bird eye level (EFSA, 2012). Poor litter quality may lead to contact dermatitis such as footpad (DE JONG; GUNNINK; VAN HARN, 2014) and breast (SOUZA et al., 2018) skin inflammatory processes or poor scores of bird soiling (FEDERICI et al., 2016). In turn, illuminance under 5 lx tends to lead birds to be less active than those subjected to at least 20 lx (RAULT et al., 2017).

Tropical or subtropical climate, as characteristic in different Brazilian regions, may also be a relevant factor concerning environmental conditions inside bird houses, which require more attention in intensive poultry production systems (COELHO et al., 2019), as external climate may interact differently with in-barn internal environmental conditions, depending on house types and seasons.

Due to the importance of the internal ambience in poultry houses, studies have been using geostatistics tools to evaluate the in-barn spatial variability of factors such as noise, temperature, relative humidity, air velocity and gas concentration, amongst others, which may influence the performance, behaviour and welfare of confined animals (MIRAGLIOTTA et al., 2006; CARVALHO et al., 2012; FERRAZ et al., 2016; DAMASCENO et al., 2018). In this context, geostatistics is an approach that allows for the spatial characterization of a variable of interest, through the study of its spatial distribution and variability within a defined area (CARVALHO et al., 2012;

YAMAMOTO; LANDIM, 2013), in our case, the internal area of broiler chicken houses. However, studies including both environmental and animal-based welfare indicators through geostatistical analysis are lacking, and when these items are used together, they may allow the adoption of strategies to improve animal welfare. The detection of location-specific welfare trends within poultry houses may support tailored corrections to welfare problems that have not yet been assessed in terms of in-barn birds and resources distribution; these trends are most likely constantly averaged off due to the poultry welfare assessment practices in use. Therefore, this research aimed to assess the spatial distribution of the prevalence of broiler chicken welfare problems regarding bird location inside the poultry house in the Southern Brazilian subtropical climate. The study considered different barn designs and seasons, to describe different welfare possibilities inside the same barn, which tends to be regarded as a single unit in most animal welfare studies and on-field monitoring practices.

4.2 MATERIAL AND METHODS

4.2.1 Animals, experimental design and housing

The participant farms were selected according to availability considering bird age, the presence of either closed or open-sided houses, respectively with either climatized or semi-climatized internal conditions (FIGURE 7). We assessed bird welfare in four closed-sided (CS) and 13 open-sided (OS) poultry houses from March to April 2019, involving the ending of summer and the beginning of autumn in Brazil (three CS and six OS houses), and from July to August 2019, corresponding to our winter (one CS and seven OS houses), thus including the assessment of 17 poultry houses in total.

FIGURE 7. VIEW OF A CLOSED-SIDED (a) AND AN OPEN-SIDED (b) POULTRY HOUSES, FROM MARCH TO AUGUST 2019, IN THE SUBTROPICAL CLIMATE.

All poultry houses were located in the West of the State of Santa Catarina, South of Brazil, and operated in an integrated system within the same company. Thus, the poultry houses studied are representative of the major poultry chain organization type as well as of the main poultry house types used in intensive systems in the country. The climate in the South of Brazil is generally characterized as subtropical (ALVARES et al., 2013). Specifically for the State of Santa Catarina during this work, the temperature ranged from 10 to 30°C during March and April, and from 6 to 22 °C during July to August 2019; relative humidity, in general, may range from 65 to 95%, from March to August (CPTEC, 2021). External environmental conditions across house designs and seasons observed for the field data collection periods are shown in TABLE 11.

TABLE 11. THE EXTERNAL AVERAGE FOR TEMPERATURE, RELATIVE HUMIDITY, AIR VELOCITY AND ILLUMINANCE, ASSESSED IN FOUR CLOSED-SIDED (CS) AND 13 OPEN-SIDED HOUSES (OS), DURING SUMMER/AUTUMN AND WINTER SEASONS 2019, IN THE SUBTROPICAL CLIMATE.

Variable	Season / House design					
	Summer / Autumn			Winter		
	CS	OS	Average	CS	OS	Average
Temperature (ºC)	25.0±2.5	26.0±3.1	25.7±2.7 b	20.0±3.5	15.7±4.3	16.2±4.4 a
Relative humidity (%)	62.0±23.9	74.1±17.1	70.1±19.8	78.4±30.5	70.9±18.5	71.8±19.1
Air velocity ($m\ s^{-1}$)	0.3±0.3	0.3±0.4	0.3±0.4	0.3±0.1	1.1±1.4	1.1±1.4
Illuminance (lx)	5142.2± 1136.6	5375.7± 1926.5	5297.8± 1671.6 b	4931.6± 515.5	4464.5± 1838.5	4873.0± 1724.1 a

Different lowercase means difference at 5% between seasons

Flock records were used to obtain general information such as the initial number of birds, number of birds at the visit, their age and breed, as well as mortality and culling rates. The first author, an animal scientist experienced in poultry welfare and the use of the Welfare Quality® protocol for poultry since 2011, performed all on-farm assessments. The participant farms raised male Cobb MX (three CS and four OS houses) and male Ross TM4 (one CS and nine OS houses). All birds received the vaccines for Newcastle, Avian Infectious Bronchitis and Gumboro diseases before leaving the hatchery. The birds were evaluated between 33 and 36 days of age, at 6.0±2.1 days before slaughter. The summary description of the studied units per house design is shown in TABLE 12.

TABLE 12. MEANS AND STANDARD ERRORS ESTIMATED FROM RESPONSES TO LEVELS OF FACTORS OF THE MAIN CHARACTERISTICS OF FOUR CLOSED-SIDED AND 13 OPEN-SIDED POULTRY HOUSES, ASSESSED DURING SUMMER/AUTUMN AND WINTER SEASONS 2019, IN THE SUBTROPICAL CLIMATE.

Variables	Season		House design	
	Summer/autumn	Winter	Closed-sided	Open-sided
Stocking density, birds/m^2	12.9 (0.092) a	12.8 (0.113) b	13.7 (0.138) a	12.0 (0.075) b
Flock size, number of birds at visit	19883 (1828) a	28922 (2764) b	25393 (3163) a	22646 (1586) a
Age at visit, d	34.2 (0.384) a	34.6 (0.466) a	34.3 (0.572) a	34.5 (0.310) a
Age at slaughter, d	39.9 (0.744) a	40.2 (0.894) a	39.2 (1.099) a	40.8 (0.599) a
Body weight at slaughter, kg	2.8 (0.045) a	2.9 (0.054) a	2.9 (0.067) a	2.8 (0.036) a
Mortality (%)	2.4 (0.410) a	3.1 (0.559) a	2.2 (0.636) a	3.3 (0.357) a
Culls (%)	1.0 (0.264) a	2.2 (0.416) b	2.1 (0.420) a	1.1 (0.261) b
Reused litter (number flocks/ litter)	5.4 (2.881) a	8.3 (3.460) a	6.2 (4.272) a	7.5 (2.310) a
Litter moisture (%)	39.7 (5.133) a	39.5 (5.860) a	37.9 (7.744) a	41.4 (3.811) a

Average pairs followed by the same lowercase letter on the line do not differ by Tukey's test, at 5% significance

All CS houses were equipped with fixed double black and silver curtains to supplement partial walls and transform them into CS houses. Exhaust fans for negative-pressure ventilation, sprinklers, illuminance controllers, heating system with automatic control, an evaporative cooling system were fitted to all CS houses. In two CS houses, air inlets were also present, allowed air flow from East to West direction.

The OS houses were semi-climatized, with laterals made of partial walls and the upper part of wire mesh covered by double yellow (10 OS houses) or blue (three OS houses) roll-up curtains. They all had positive-pressure mechanical ventilation by fans distributed from East to West direction with at least three fan lines and a minimum of two fans per line, sprinklers, and natural complemented with artificial lighting. All poultry houses were built in East-West orientation, had 1200 m^2 in size, equipped with illuminance by either light-emitting diode lamps (LED), incandescent, fluorescent or mixed-light types within the same unit, and wood shavings as litter. Automatic feeders in a ratio of 1:40 feed per birds were installed at every 50 cm; nipple drinker ratio was 1:12 birds, and nipples were installed every at 20 cm. The lighting program consisted at least 6 h of darkness, from the bird age of 22 d-old onwards. Background information on illuminance is that there was is no recommendation for minimum illuminance in OS, as this house type is not considered critical in terms of light availability for the animals; however, for CS houses there is a recommendation to provide a light intensity of at least 20 lx. The referenced recommendation in terms of air velocity was >1.8 m s^{-1} for birds from 29 d age. The time visits to collect data regarding animal-based and environmental indicators varied between 08:15 AM and 05:40 PM. The assessment started between 8:15 and 10:00 AM and ended at around 3:00 PM (one CS and nine OS houses); in seven other farms, the assessment started at 11:00 AM and ended around 5:40 PM (three CS and four OS houses). On farms with more than one poultry house, only one of them was randomly selected for data collection.

4.2.2 Environmental indicators

In-barn environmental indicators were collected simultaneously in all units, to characterize the indoor living conditions of the broiler chickens (TABLE 13).

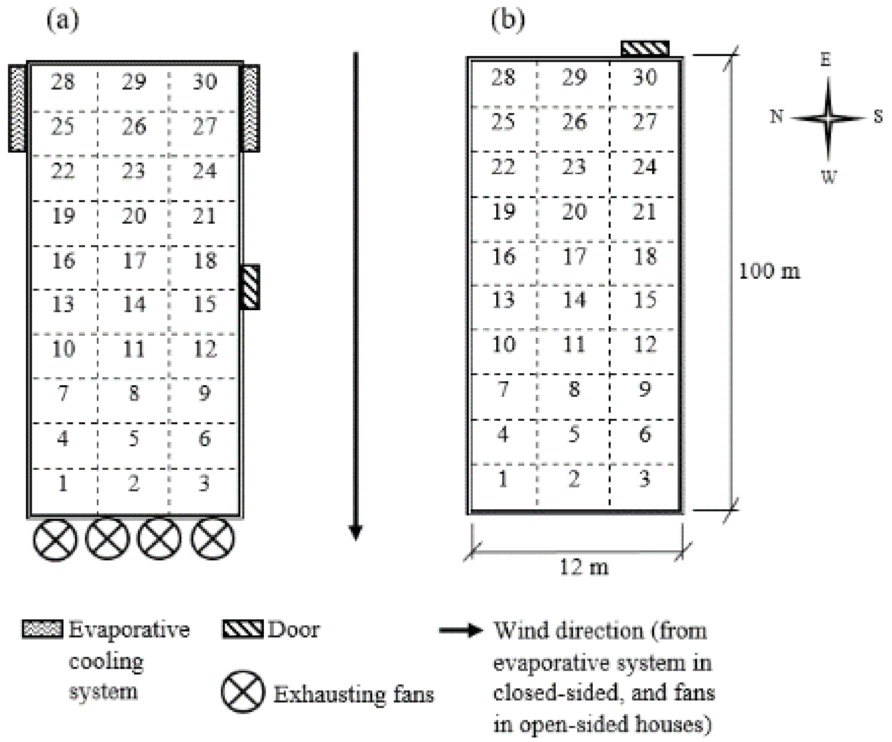
TABLE 13. THE ESTIMATED MEAN AND STANDARD ERROR FOR INDOOR TEMPERATURE, RELATIVE HUMIDITY, AIR VELOCITY, ILLUMINANCE, AMMONIA (NH_3), AND CARBON DIOXIDE (CO_2) CONCENTRATIONS, FOR COMBINATIONS OF THE LEVELS OF THE SEASON (SUMMER/AUTUMN AND WINTER) AND HOUSE TYPE (OPEN- AND CLOSED-SIDED), ASSESSED IN 2019, IN THE SUBTROPICAL CLIMATE.

Variables	Season / House design	Closed-sided	Open-sided
Temperature (°C)	Summer/autumn	26.7 (0.6) aA	27.1 (0.4) aA
	Winter	25.9 (0.5) aA	21.8 (0.4) aB
Relative humidity (%)	Summer/autumn	62.0 (4.5) bB	75.5 (4.2) aA
	Winter	77.9 (4.4) aA	70.7 (4.2) bB
Air velocity (m s^{-1})	Summer/autumn	1.8 (0.2) aB	1.0 (0.1) bA
	Winter	2.4 (0.2) aA	0.2 (0.2) bB
Illuminance (lx)	Summer/autumn	4.4 (1.3) bA	145.3 (39.9) aA
	Winter	6.7 (2.4) bA	119.8 (32.7) aA
NH_3 (ppm)	Summer/autumn	10.6 (2.4) aA	9.2 (2.3) aA
	Winter	10.4 (3.1) bB	16.6 (2.2) aA
CO_2 (ppm)	Summer/autumn	1090.7 (1.1) aB	837.8 (1.1) bB
	Winter	1551.8 (1.1) bA	2082.8 (1.1) aA

Pairs of averages on the probability scale, followed by the same uppercase letter in the column and lowercase in the row do not differ by Tukey's test at 5% significance

Temperature, relative humidity, illuminance, NH_3 and CO_2 concentrations were assessed at bird level and in each 30 equidistant locations (FIGURE 8). Temperature, relative humidity and CO_2 concentration were assessed with Akso AZ 77535, Hong Kong, China, as well as the external temperatures at the beginning and end of data collection. The features of Akso indicate that for temperature, the equipment range is from -10 to 60 ºC and accuracy of ±0.6 ºC; measurable relative humidity ranges from 0.1 to 99.9%, with an accuracy of ±3% (at 25 ºC, 10 to 90%, others ±5%); and CO_2 from 0 to 9999 ppm with an accuracy of ±30 ppm (0-5000 ppm). Air velocity and illuminance were measured with LM 8000A, with measurable ranges and accuracies from 0.4 to 30 m s^{-1} and 0 to 20.000 lx, and ±3% and 5%, respectively. Ammonia concentration was assessed, at bird level, with SP2nd NH_3 Portable Single-Gas Ammonia Detector, with a measurable range from 0 to 100 ppm, accuracy 5%.

FIGURE 8. VIEW OF 30 EQUIDISTANT LOCATIONS FOR ENVIRONMENTAL AND ANIMAL-BASED INDICATORS, ASSESSED IN FOUR CLOSED-SIDED (a) AND 13 OPEN-SIDED (b) HOUSES, DURING SUMMER/AUTUMN, AND WINTER SEASONS 2019, IN THE SUBTROPICAL CLIMATE.



For the litter moisture analysis, approximately 400 g of litter were collected at 12 locations per house, avoiding areas near or below the feeders and drinkers. These samples were packed in identified plastic bags and sent for analysis. Following Tedesco et al. (1995) for the measurement of litter moisture, 20 to 30 g of litter samples were homogenized and placed in a forced ventilation oven at 65-70 °C for 24 or 48h, or until no change in weight was observed with increasing drying time.

4.2.3 Animal-based indicators

Animal-based indicators assessed were contact dermatitis on the breast and abdominal areas, bird soiling, footpad dermatitis and hock burn, all observed in the same sample of 150 birds per flock (WELFARE QUALITY®, 2009; SOUZA et al., 2018). Lameness was assessed in a different sample of 150 birds (WELFARE QUALITY®, 2009). In general, on-farm animal-based indicators vary from scores 0 to 3, 4 or 5, with 0 denoting the absence of the problem and the highest score indicating the most severe situation. However, for better data treatment to statistical and geostatistical analysis, the results were binarized between absence (0) and presence (1), as follows: for contact dermatitis on the breast and abdominal areas, scores 0 and 1 were considered dermatitis absence, and 2 and 3 presence; for bird soiling, 0+1=absence and 2+3=presence; for footpad dermatitis, 0+1=absence and 2+3+4=presence; for hock burn, 0+1=absence and 2+3+4=presence; and for lameness, 0+1+2=absence and 3+4+5=presence. For the assessments, the house was divided into 30 equidistant locations (FIGURE 8), with at least 10 randomly selected birds per location. Five birds were randomly selected and assessed for contact dermatitis on the breast and abdominal areas, bird soiling, footpad dermatitis and hock burn and another five for lameness, totalizing 300 birds assessed per flock. The data collections started from point 1 to 30, starting from the West to the East side of the barn, which is equivalent to progressive assessment locations from exhaust fans to evaporative cooling system in CS houses and in the same direction as the air coming from the fans in OS houses.

4.2.4 Statistical analysis

4.2.4.1 General analyses of environmental and animal-based indicators

Differences in external temperature, relative humidity, air velocity and illuminance were analyzed by t-test for two independent samples. Linear regression models were fitted to the data, considering season, house type and the effect of interaction between these factors as predictive variables. The house effect was incorporated into the models using random outcomes, which assumed a normal distribution with zero mean and constant variance (σ^2). Illuminance and CO_2 variables were tested with the transformation of the Box-Cox family, being the natural logarithm more appropriate to improve the quality of fit of these models. For stocking density data, age at the visit, age at slaughter, body weight at slaughter, flock size, mortality, culls, reused litter and litter moisture, the interaction effect between factors was not tested because there was only one observation in the combination of the levels of the winter season in CS house. Due to a non-constant residual pattern, a diagonal matrix of weights was inserted in the models. The weights correspond to the inverse of the response variance, also calculated for each house. For the variable flock size, a generalized linear model with Poisson distribution and logarithmic link function was adjusted to the data. However, a problem of overdispersion was observed, for which a quasipoisson model was better fitted. For animal-based indicators, a generalized linear model was used, considering the Bernoulli probability distribution and logit link function.

All conclusions for the data set analyses were based on a significance level of α equal to 5%, through the program R (R CORE TEAM, 2019). The generalized linear models were fitted with the package base, while the models with random effects were fitted with the package lme4 (BATES et al., 2015). The contrasts estimated in the package were expressed in means (LENTH, 2020) and the Tukey test was used for multiple comparisons of means. For models with Bernoulli distribution, the difference between the levels of the factors was estimated on the odds ratio scale. The fit quality of the tested models was assessed using half-normal plots available in the hnp package (MORAL; HINDE; DEMÉTRIO, 2017).

4.2.4.2 Geostatistical modelling

In this study, we used a geostatistical approach to the continuous responses (relative humidity, temperature, air velocity, NH_3 and CO_2 concentrations, and illuminance), and to the binary responses (occurrence or not of bird soiling, footpad dermatitis, hock burn and lameness). In general, our analyses consisted of a sequence of four steps:

Step 1 - Fitting, through the least-squares method, the semivariogram proposed by Matheron (1962):

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [Z(xi) - Z(xi + h)]^2,$$

where:

$\gamma(h)$ = semivariance and sampling obtained through the achieved results;

N (h) = the number of experimental pairs of observations $Z(x_i)$ and $Z(x_i + h)$ separated by a distance (*h)*;

This a descriptive step, but also provides initial values for the model parameters, which were defined in step 2.

Step 2: In this step, we considered the following geostatistical model (DIGGLE et al., 1998) to describe the continuous responses:

$$Y_i = \mu(x_i) + S(x_i) + \epsilon_{i,} i = l, \dots, n$$

Where Y_i is the observed value on the response variable at the position x_i, $\mu(x_i)$ is the fixed part with no covariates and ϵ_i is a mutually independent-zero-mean Gaussian variable.

In the case of binary responses, we used the generalized linear geostatistical model (GIORGI; DIGGLE, 2016). This approach consists in modifying model 1 by adding a “logit” function to link the response variable Y_i and the fixed part $\mu(x_i)$ as follows:

$$E[Y_i \vee S(x_i), \epsilon_i] = m_i p_i$$

$$log\left(\frac{p_i}{1-p_i}\right) = \mu(x_i) + S(x_i) + \epsilon_{i,} i = l \ldots, n$$

Where Y_i are positive counts, m_i is the binomial denominators and $p_i = Y_i/m_i$. In both cases $S(x_i)$ is defined as a stationary isotropic Gaussian process with variance σ^2 and a candidate correlation function.

We tested six different covariance functions (linear, exponential, cubic, circular, spherical and Gaussian) for the models with continuous responses. On the other hand, in the case of models with discrete responses, only the Matérn family was considered, differentiating the models by varying the value of the shape parameter kappa.

For the continuous responses, the model fitting was performed using ordinary maximum likelihood estimation while for the binary responses the model fitting was performed by the Monte Carlo maximum likelihood method described in Giorgi, Diggle (2016).

Step 3: The fitted models were selected using the Akaike Information Criterion or AIC (AKAIKE, 1974) and since no covariate was considered for the fixed part of the model $\mu(x_i)$, the model selection was limited to the comparison between the different covariance functions.

Step 4: In this step, the selected models were used to perform spatial predictions (kriging) on a certain grid of x, y coordinates. These predicted values were then plotted to generate the maps of the spatial results of the response variables.

To analyze the degree of spatial dependence (DSD) the classification used was strong (DSD≥75%), moderate (25%<DSD<75%) and weak (DSD≤25%; CAMBARDELLA et al., 1994). The analysis was performed using the R software (R CORE TEAM, 2019) with the libraries geoR (JUNIOR RIBEIRO; DIGGLE, 2001) and PrevMap (GIORGI; DIGGLE, 2016).

4.2.5 Ethical approval

This work was approved by the Animal Use Ethics Committee of the Agricultural Campus (No 046/2018; July 5th, 2018), Federal University of Paraná (ANNEX I, II).

4.3 RESULTS

4.3.1 Environmental indicators

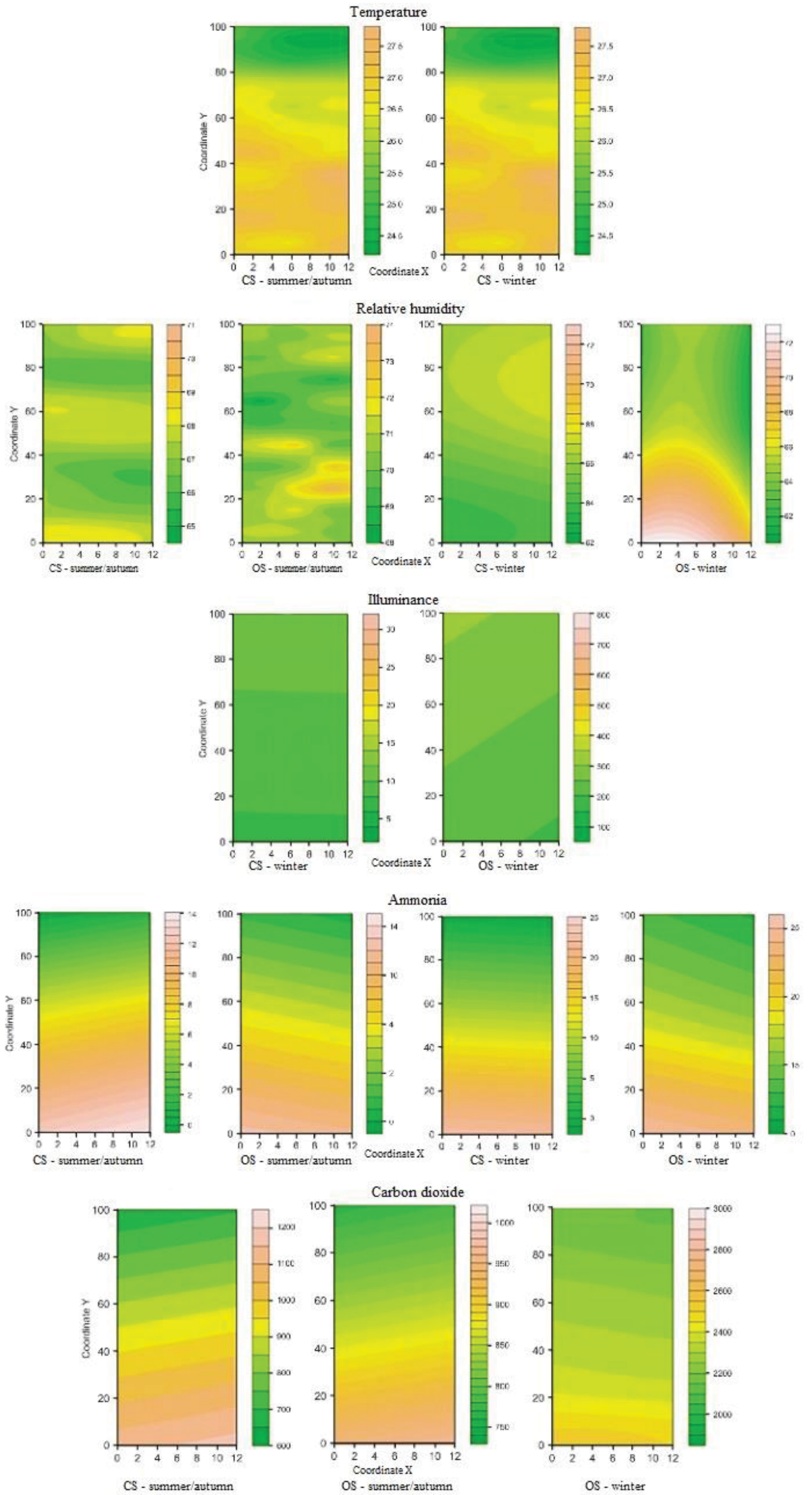
The semivariograms for environmental assessments were predominantly fitted to the cubic model, followed by others based on linear, exponential, circular and Gaussian distribution (TABLE 14). Most of the environmental measurements showed spatial dependence, varying between strong (DSD of 100%) and moderate (DSD from 39% to 51%), also with ranges varying from 1.0 to 18120.3 m. Thus, the possible use of larger distances between sampling may be inferred, especially for indicators that showed strong spatial dependence. No spatial dependence was observed for air velocity, and this indicator was not included in TABLE 14.

Kriging maps (FIGURE 9) showed heterogeneity for environmental indicators. In addition, when poultry house types and seasons were compared, some maps showed patterns of higher prevalence of certain problems for specific locations within the barn.

TABLE 14. ESTIMATED PARAMETERS OF GEOSTATISTICAL MODELS FOR TEMPERATURE (°C), RELATIVE HUMIDITY (%), AIR VELOCITY (M S-[1]), ILLUMINANCE (LX), AMMONIA (NH_3, PPM), AND CARBON DIOXIDE (CO_2, PPM) CONCENTRATIONS, ASSESSED IN FOUR CLOSED-SIDED (CS) AND 13 OPEN-SIDED (OS) HOUSES, DURING SUMMER/AUTUMN AND WINTER SEASONS 2019, IN THE SUBTROPICAL CLIMATE.

Season	House design		Environmental measurements				
			Temperature	Relative humidity	Illuminance	NH_3	CO_2
Summer /Autumn	CS	Model	Circular	Cubic	Exponential	Cubic	Cubic
		Nugget effect	1.7	0.1	0.0	1.0	2940.6
		Sill	317.3	2.0	0.0	53323.2	$2.6 . 10^7$
		Range (m)	20.6	18.8	3.0	6850.2	3958.2
		DSD (%)	100.0	51.0	-	100.0	100.0
		Classification	Strong	Moderate	SD	Strong	Strong
	OS	Model	Cubic	Exponential	Linear	Cubic	Cubic
		Nugget effect	0.0	0.2	0.0	0.6	794.9
		Sill	0.0	2.0	0.0	40100.9	$1.3 . 10^7$
		Range (m)	8.0	4.5	1.0	11250.4	6693.3
		DSD (%)	-	90.0	-	100.0	100.0
		Classification	SD	Strong	SD	Strong	Strong
Winter	CS	Model	Cubic	Cubic	Cubic	Cubic	Linear
		Nugget effect	0.6	5.7	40.3	5.7	$1.2.10^5$
		Sill	2326.6	9.3	$6.9.10^4$	$1.1 . 10^5$	$1.2 . 10^5$
		Range (m)	5408.0	94.8	18120.3	5128.3	1.0
		DSD (%)	100.0	39.0	100.0	100.0	-
		Classification	Strong	Moderate	Strong	Strong	SD
	OS	Model	Cubic	Cubic	Gaussian	Cubic	Linear
		Nugget effect	0.0	$4.8.10^7$	$2.2.10^4$	4.4	2133.2
		Sill	0.0	$5.1.10^7$	$1.2. 10^7$	1066.6	3608.0
		Range (m)	40.5	28.1	1579.3	971.6	1.0
		DSD (%)	-	7.0	100.0	100.0	41.0
		Classification	SD	Weak	Strong	Strong	Moderate

FIGURE 9. SPATIAL DISTRIBUTION OF TEMPERATURE (T ºC), RELATIVE HUMIDITY (%), ILLUMINANCE (LX), AMMONIA (PPM), AND CARBON DIOXIDE CONCENTRATIONS, ASSESSED IN FOUR CLOSED-SIDED AND 13 OPEN-SIDED HOUSES, DURING SUMMER/AUTUMN AND WINTER SEASONS 2019, IN THE SUBTROPICAL CLIMATE.



Higher temperatures, NH_3 and CO_2 concentrations were observed from the West to the central part of the houses. However, despite the strong spatial dependence, the kriging map showed a homogeneous spatial distribution of illuminance. Results for relative humidity did not follow this pattern, and their location varied across house types and seasons.

4.3.2 Animal-based indicators

Results for contact dermatitis on the breast and abdominal areas did not show an interaction effect for seasons and house types (P = 0.957), nor a main effect for house types (P = 0.128). However, there was a difference in the dermatitis probability depending on the season and house types. For footpad dermatitis, bird soiling, hock burn and lameness, a significant interaction between season and house types was observed (TABLE 15).

TABLE 15. THE ESTIMATED MEAN AND STANDARD ERROR FOR THE PRESENCE OF BIRD SOILING, FOOTPAD DERMATITIS, HOCK BURN, AND LAMENESS, FOR THE COMBINATIONS OF FACTOR LEVELS SUCH AS SEASONS (SUMMER/AUTUMN AND WINTER), AND HOUSE TYPES (OPEN- AND CLOSED-SIDED), ASSESSED IN 2019, IN THE SUBTROPICAL CLIMATE.

Variables	Season / House design	Closed-sided	Open-sided
Footpad dermatitis	Summer/autumn	34.0% (2.2%) aA	32.7% (1.6%) aB
	Winter	15.3% (2.9%) bB	51.0% (1.5%) aA
Bird soiling	Summer/autumn	28.7% (2.1%) aA	10.7% (1.0%) bB
	Winter	2.0% (1.1%) aB	19.8% (1.2%) aA
Hock burn	Summer/autumn	6.9% (1.2%) aA	0.8% (0.3%) bA
	Winter	1.3% (0.9%) aB	6.0% (0.7%) aA
Lameness	Summer/autumn	57.8% (2.3%) aA	50.9% (1.7%) bB
	Winter	78.0% (3.4%) aA	51.5% (1.5%) bA

Pairs of means on the probability scale followed by the same uppercase letter in the column and lowercase in the row do not differ at 5% significance.

Geostatistics results for animal-based indicators showed more spatial dependence for footpad dermatitis, followed by bird soiling, hock burn and lameness, which the classification varied from moderate to strong, according to house design and season (TABLE 16). Most of the results did not show spatial dependence due to a large volume of the zero value (absence) compared with 1 (presence) of the animal welfare problems. The range extrapolates the size of the houses, varying from 4.91 to

945.11 m, due to the use of the Matérn model, which fitted better for experimental data and allowed binary variable analysis. There was no classification of spatial dependence for contact dermatitis on the breast and abdominal areas, and this indicator was not included in TABLE 16.

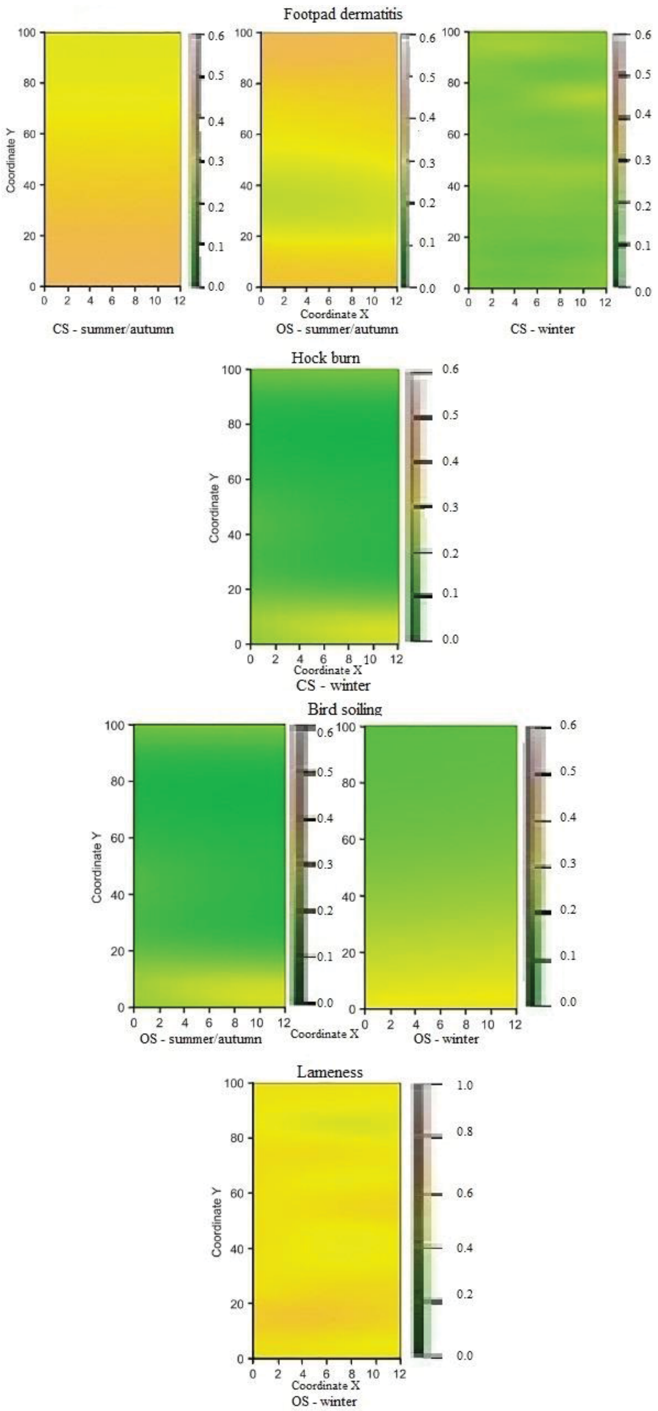
TABLE 16. ESTIMATED PARAMETERS OF THE MODEL WITH MATÉRN COVARIANCE FUNCTION AND KAPPA=10 FOR ABSENCE AND PRESENCE FOR FOOTPAD DERMATITIS, CONTACT DERMATITIS ON THE BREAST AND ABDOMINAL AREAS, BIRD SOILING, HOCK BURN, AND LAMENESS, ASSESSED IN FOUR CLOSED-SIDED (CS) AND 13 OPEN-SIDED (OS) POULTRY HOUSES, DURING SUMMER/AUTUMN AND WINTER SEASONS 2019, IN THE SUBTROPICAL CLIMATE.

Season	House design		Health indicators			
			Footpad dermatitis	Bird soiling	Hock burn	Lameness
Summer/ Autumn	CS	Model	Matérn	-	Matérn	-
		Nugget effect*	-2.3	-	-0.8	-
		Sill*	-5.2	-	1.2	-
		Range (m)	109.7	-	945.1	-
		DSD (%)	43.0	-	100.0	-
		Classification	Moderate	SD	Strong	
	OS	Model	Matérn	Matérn	-	-
		Nugget effect*	-2.5	-1.6	-	-
		Sill*	-4.7	-2.2	-	-
		Range (m)	13.3	14.9	-	-
		DSD (%)	47.0	29.0	-	-
		Classification	Moderate	Moderate	SD	SD
Winter	CS	Model	Matérn	-	-	-
		Nugget effect*	-0.6	-	-	-
		Sill*	-10.6	-	-	-
		Range (m)	4.9	-	-	-
		DSD (%)	94.0	-	-	-
		Classification	Strong	SD	SD	SD
	OS	Model	-	Matérn	-	Matérn
		Nugget effect*	-	-1.9	-	-4.1
		Sill*	-	-3.1	-	-7.2
		Range (m)	-	754.5	-	2.2
		DSD (%)	-	39.0	-	44.0
		Classification	SD	Moderate	SD	Moderate

*Logarithmic scale; C_0 = Nugget effect; Sill (C_0+C_1); DSD = Degree of spatial dependence (C_1/C_0 + C_1) x 100; SD = without spatial dependence

The spatial distributions for animal-based indicators were heterogeneous (FIGURE 10). Birds in the West part of CS and OS houses faced higher prevalence for footpad dermatitis, hock burn and bird soiling during summer/autumn and winter season, with specific patterns in terms of spatial distribution for these indicators.

FIGURE 10. SPATIAL DISTRIBUTION OF THE ABSENCE OR PRESENCE OF FOOTPAD DERMATITIS, HOCK BURN, BIRD SOILING, AND LAMENESS ASSESSED IN FOUR CLOSED-SIDED AND 13 OPEN-SIDED HOUSES, DURING SUMMER/AUTUMN AND WINTER SEASONS 2019, IN THE SUBTROPICAL CLIMATE.



4.4 DISCUSSION

In general, problems from both environmental and animal-based indicators showed prevalence heterogeneity for different house types and seasons. From the West to the central area of both CS and OS houses, there were higher, i.e. worse, values for three environmental indicators (temperature, NH_3 and CO_2 concentrations) and three animal-based indicators (footpad dermatitis, hock burn and bird soiling).

Regarding environmental indicators, most of the time, values for temperature, NH_3 and CO_2 concentrations in the West region of the house were further from the recommended limits for the welfare of chickens. Temperature averages, for example, exceeded 20ºC, the limit recommended for 6 wk-old chickens (ABREU; ABREU, 2011); relative humidity exceeded the limit of 70% (EFSA, 2012). The Westside of CS houses was fitted with exhaust fans and in OS houses the West was the direction of positive-pressure mechanical ventilation by fans. Thus, a distribution showing regions with higher prevalence of certain problems was expected. According to Nääs et al. (2014), both temperature and relative humidity may directly impact animal welfare. Moreover, our results demonstrated that the location of each bird inside the barn may also directly influence their welfare, as relative humidity exceeded the limit of 70% at least once for each of the two house types and seasons.

Regarding the seasons, birds reared in both houses designs were subjected to inadequate thermal conditions. Although temperature maps for OS were not relevant, the average temperature as measured during visits, show that this environmental indicator, for both house types, and especially during summer/autumn, tended to be above the limit. Higher temperature and lower air quality have been reported in the direction of the exhaust fans in CS houses (CEMEK; KUCUKTOPCU; DEMIR, 2016; COELHO et al., 2019; DAMASCENO et al., 2019). According to Lima, Silva (2019), birds reared in OS houses may experience better air quality, but are more susceptible to thermal stress.

Curtain management is likely important contributors to better gas dispersion in OS houses. Nevertheless, to avoid heat loss from the internal to the external environment during winter, curtain lowering is generally reduced, which may contribute to the increase of NH_3 and CO_2 concentrations in OS when compared to CS houses (SANS et al., 2021b *in press*). In OS houses, even though lowered curtains seem an efficient tool in reducing NH_3 concentration (NÄÄS et al., 2007; LIMA et al., 2020), there seems to be a conflict in curtain management, especially during wintertime, and this may be one of the main causes leading to worse results regarding the indoor environment in this house design.

It is also important to inspect the correct distribution of the fans, throughout the house, and if they are functioning properly, to ensure air renewal. If the ventilation rate is not adequate, it may negatively influence the results for both environmental and animal-based indicators, such as poor air quality and panting behaviour (SANS et al., 2021a; 2021b, *in press*). In our study, we observed air velocity ranging from 1.8 to 2.4 m s^{-1} in CS and 0.2 to 1.0 in OS houses. Thus, only in CS houses values closer to the recommended 1.7-3.0 m s^{-1} for birds over 28 d (COBB, 2012) were observed. This is additionally related to the fact that equipment in CS barns provides a higher ventilation rate when compared to the positive ventilation equipment in OS barns (LIMA et al., 2011). However, in both house types, there are specific in-barn areas with clear difficulties in the maintenance of good air quality. In OS houses, areas with poor air quality are related to the inadequate positioning or functioning of the fans, the low number of fans per house, sprinklers or inefficient curtain management. In CS houses, the existence of a single air intake makes circulation difficult, compromising the environmental quality of the house. In addition, there is a lack of training on environmental control and eventual mechanical problems of controllers, exhaust fans or evaporative cooling systems. Bird welfare may be compromised in both house types, when there is no observation of bird behavior, which is relevant to guide management practices. All of which may influence the temperature and air distribution within the barns.

Overall, the use of both curtains and fans may be advantageous, as curtains extend throughout the house and on both sides. In CS houses, the optimal ventilation rates are more easily achieved, and this is an advantage for this house type. However, because the fresh air enters through the evaporative cooling systems, it is possible that when it reaches the opposite direction near the exhaust fans, the air may be saturated with gases and higher in relative humidity. This is likely an additional reason for the West area of the barns showing lower air quality.

Illuminance spatial distribution showed fewer differences as compared with temperature, NH_3 and CO_2 concentrations, and it was not possible to characterize specific areas with higher or lower illuminance. The small difference observed for the distribution of light across the whole barn, in both house types, may occur due to different types of lamp and, in OS, to the added variation of natural light during the day. In general, the farmers seek to standardize lighting in houses using LED bulbs, due to their lower energy consumption, longer lamp life and better luminous intensity (NUNES et al., 2013; RIBEIRO et al., 2016). Nonetheless, this pattern may change as lamp replacements become necessary, considering that LED lamps are more expensive than incandescent or fluorescent light bulbs.

The main issue regarding lighting was observed in CS houses, in which illuminance was severely restricted, below the minimum recommendations for bird welfare (20 lx; EFSA, 2012). The higher illuminance observed in OS houses was due to the open lateral walls that allowed for the entry of sunlight, even when curtains were partially closed or handled less often. Low illuminance may negatively influence locomotor activity and behavioural repertoire of birds and induce leg disorders (DEEP et al., 2010; EFSA, 2012; RAULT et al., 2017). As exemplified by illuminance, although the current trend in the Brazilian poultry industry is to increase the proportion of CS houses, this seems incoherent with some animal welfare issues and such move appears to differ from resolutions adopted in European countries (SOUZA; MOLENTO, 2015). Therefore, the discussion regarding the best types of broiler chicken barn benefits from the consideration of animal welfare, which seems essential to avoid compromising the basic needs of the birds, no matter where they are located inside the barns.

The kriging maps for the results of animal-based indicators, such as footpad dermatitis, hock burn and bird soiling, showed a tendency of higher prevalence of problems for animals located near the Western end of the houses. The prevalence of contact dermatitis, such as footpad dermatitis, was high during summer/autumn in both CS and OS houses, and the prevalence increased in OS houses during winter. However, Louton et al. (2018) did not observe, for any season, a significant effect of open- *vs* closed-houses for footpad dermatitis and hock burn. That means the season effect should be considered, because in some cases, may interfere on chickens' welfare. Garcia et al. (2019) reported no evidence of different prevalences of footpad dermatitis amongst birds in CS and OS houses, but they suggested a probable lower risk for birds in CS houses. On the other hand, Rovaris et al. (2014) observed a higher prevalence of foot callus in birds raised in CS than in OS houses, during a full year of evaluation. Even though footpad dermatitis has been identified as a critical point for years (BESSEI, 2006), it remains a recurrent animal welfare problem and its prevalence seem to vary according to the location of the bird in the barn. Our results for hock burn showed an alternation in prevalence, according to house type and season, with higher prevalence during summer/autumn in CS and during the winter in OS houses. A more detailed understanding of all relevant factors seems essential, as contact dermatitis is the most common lesion observed in poultry, with high potential for compromising bird welfare (MELUZZI; SIRRI, 2009; SHEPHERD; FAIRCHILD, 2010; DE JONG; GUNNINK; VAN HARN, 2014).

Results for bird soiling suggest the absence of good environmental conditions within both house types, coherent with the barn area of highest prevalence for hock burn, footpad dermatitis, NH_3 and CO_2 concentrations, i.e. close the West end. The ammonia, for example, is corrosive and may be related to the higher prevalence of contact dermatitis, especially considering its direct contact with the skin of birds (NASEEMAND KING, 2018). According to Federici et al. (2016), there is a positive correlation between litter quality and bird soiling, suggesting that assessing both indicators may show both feather cleanliness and whether the environment provides adequate raising conditions in terms of a clean and comfortable place to rest.

Souza et al. (2018) also reported a moderate correlation between litter quality, bird soiling and contact dermatitis on the breast and abdominal areas, assessed in OS houses in Southern Brazil, while Granquist et al. (2019) associated low litter quality with worse gait scores associated with reduced bird soiling score. In general, the evaporative cooling system side of CS houses is characterized by higher relative humidity (DAMASCENO et al., 2019) and, in case of values regarding relative humidity above recommended standards, breast and foot callus prevalence tends to be worse (GARCIA et al., 2018). Nonetheless, our results showed a higher prevalence of this lesion in the direction of the exhaust fans. The higher temperatures and worse air quality usually observed next to exhaust fans (COELHO et al., 2019; DAMASCENO et al., 2019) may be related to a higher prevalence of contact dermatitis in this area of the house.

The spatial dependence of lameness was moderate in CS and OS houses during summer/autumn, and strong in CS houses during winter. In general, this problem was observed in a large number of birds, regardless of their location inside the barn, and increased significantly during winter in CS houses. However, Louton et al. (2018) did not find a difference for lameness between closed- and open-sided. According to our results, the prevalence of this problem was more than 50.0%, reaching 78.0% of the birds in CS houses during winter, which reveals a major welfare problem for birds in industrial intensive poultry systems, regardless of the kriging map results. Lameness promotes significant physiological challenge to birds by pain (EFSA, 2012; GRANQUIST et al., 2019), changing their behaviour by keeping them more inactive and decreasing the number of visits to feeders (WEEKS et al., 2000). Granquist et al. (2019) observed a correlation between lameness and the increased prevalence of hock burn and footpad dermatitis. The same authors did not observe the occurrence of lameness coinciding strictly with the humidity of the litter, which reinforces its multifactorial origins. The environmental characteristics and other factors may interfere with the health of bird legs, such as growth rate, bird age during assessment, simultaneous diseases, nutrition, live weight and genotype, increasing the prevalence of more severe lameness scores (KESTIN et al., 2001; BESSEI, 2006; KNOWLES et al., 2008). Some measures can be implemented to reduce the prevalence of lameness. However, they frequently involve a decrease in the growth rate and production efficiency, making an important debate about the viability of these actions (KNOWLES et al., 2008), in other words, an opportunity for the reflection on what is the priority issue when animal welfare and economic gains are in conflict.

It was not possible to compare the relative humidity and litter quality across different collection sites, as they were mixed to compose a single sample per barn, which was then sent to the laboratory. Both environmental conditions may influence the prevalence of animal-based indicators, added nutrition, sex, body size, stocking density, and genetic (MAYNE, 2005; HASLAM et al., 2007; SHEPHERD; FAIRCHILD, 2010; DE JONG; GUNNINK; VAN HARN, 2014). However, in the Western area it was observed higher prevalence of animal-based problems, suggesting poorer litter quality than other areas. It is important to avoid the accumulation of waste arising from reused litter, which may tend to lead to the generation of gases, due to microbial decomposition, or with litter moisture, which may affect the walking ability (SAKAMOTO; BENINCASA; SILVA, 2020). There is a recommendation for the litter not to be reused for more than six consecutive flocks (CAMPOS et al., 2018); however, the litter should provide adequate absorption, and a clean and comfortable place for birds to rest. Even then, further challenging of this recommendation seems warranted, as rearing birds on the excreta of animals that previously lived inside the barn is an unseen practice in other contexts. Our results showed that the litter reuse varies from 5.4 to 8.3 flocks/litter, indicating more attention is needed for this practice. Minimally, for each new flock placed there should be a new litter quality assessment.

The litter moisture is another indicator that, combined with its reuse frequency, may influence on birds' welfare. Results showed that in both season and house types, litter moisture were always above 37%, and gases concentration were also higher in specific areas. Taira et al. (2014) considered wet those litter with moistures values above 30% may be considered wet, fact that may lead birds to have dermatitis. In this study, both reuse litter and moisture may have contributed to the prevalence of contact dermatitis, especially in West area.

In this present study, an additional factor influencing the prevalence of contact dermatitis was identified: the location of the bird inside the barn, as it relates with different in-barn environmental conditions. This fact is likely aggravated by the difficulties to move around the barn that birds face, such as low illuminance that may decrease the birds' activity (DAVIS et al., 1999; RAULT et al., 2017), increasing leg problems as birds age, and high stocking density (BESSEI, 2006). The season may also be an important factor to be considered regarding footpad dermatitis prevalence, with the winter reported as the season of its highest prevalence (HASLAM et al., 2007; SHEPHERD; FAIRCHILD, 2010). Our results did not show a difference for footpad dermatitis prevalence during summer/autumn, but during winter this injury increased significantly in OS and decreased in CS house. These opposite results may be a reflection of the better environmental conditions of air velocity, NH_3 and CO_2 concentrations observed in CS than OS houses during winter, which may have influenced litter quality, and consequently, the prevalence of the dermatitis. Overall, our results showed a high percentage of birds suffering from footpad dermatitis throughout the year, which was influenced by bird location in the barn and was different for each house type as well as influenced by the season of the year.

The internal environment of the houses is important for chicken welfare and the more tools, such as in-barn geostatistics, are available to detect issues to be improved, the more we may improve bird life quality. This contributes to the science of animal welfare and helps responding to some of the societal demands in animal ethics. Most of all, by improving our abilities to diagnose animal welfare problems, we increase our ability to alleviate their suffering.

4.5 CONCLUSION

Spatial distribution heterogeneity was observed for the prevalence of both environmental and animal-based problems. There was a systematic spatial distribution of worse welfare problems heading from the middle of the house towards the West end of both house types, for three environmental (temperature, NH_3 and CO_2 concentrations) and three animal-based welfare indicators (hock burn, bird soiling, and footpad dermatitis). Two factors were evenly distributed inside the barns, illuminance, which was very restricted in CS as compared to OS houses, and lameness, with high prevalence in both house types. Although broiler chickens experienced discomfort in both house types, the kriging maps allowed for the identification of worse welfare problems in the West direction, which in CS houses means near exhaust fans and in OS houses the direction of positive-pressure mechanical ventilation by fans. Our results show that attention is needed for the variation of bird welfare conditions inside each barn, and allow for the adoption of strategies to spread best conditions throughout the internal barn area in both house designs. Such strategies include observing the environmental conditions of the geographical region when planning house types, the optimized handling of curtains and training of livestock people regarding the use of environmental controllers. Principally, our findings on in-barn bird welfare heterogeneity suggest the relevance of constant bird welfare monitoring in key locations within the barns, through the use of devices to monitor the environmental-based indicators, such as temperature and gas concentrations, and the use of animal-based indicators, such as the observation of bird soiling and prevalence of dermatitis.

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5. FROM THE POINT OF VIEW OF THE CHICKENS: WHAT DIFFERENCE DOES A WINDOW MAKE?

RESUMO

A luz é um importante fator ambiental em vários aspectos para os frangos de corte, como no comportamento e fisiologia, e o seu bem-estar pode ser comprometido, caso as aves sejam mantidas em uma baixa iluminância. O objetivo deste trabalho foi investigar o que os frangos de corte preferem quando a eles é oferecida livre escolha para acessar ambientes, dentro do galpão, com disponibilidade exclusiva de luz artificial e outro ambiente com disponibilidade de luz natural fornecida por meio de janelas com vidros. Oitenta e cinco frangos de corte machos, de um dia de idade, linhagem Cobb, foram divididos em 10 unidades experimentais. O galpão foi dividido longitudinalmente, sendo um lado com um ambiente escuro, sem janelas e disponibilidade exclusiva de luz artificial (OAL), e do outro lado, um ambiente com janelas distribuídas em sua lateral, recebendo tanto a luz natural quanto artificial (NAL); as unidades experimentais foram construídas transversalmente, sendo que metade de cada unidade tinha disponível o acesso ao ambiente OAL e a outra metade ao ambiente NAL. Dez lâmpadas brancas do tipo LED foram disponibilizadas por todo o galpão, em ambos os ambientes. As aves escolheram livremente em que lado preferiram permanecer. Indicadores ambientais internos e condições ambientais externas como temperatura, umidade relativa, velocidade do ar, concentração de amônia e iluminância foram monitorados. A preferência dos frangos de corte foi avaliada pelo registro do número de aves presente em cada ambiente e repertório comportamental, registrado a cada três dias, de 9 a 36 de idade das aves (totalizando 10 d de observações). A comparação entre os ambientes OAL e NAL em relação aos indicadores ambientais internos foram efetuadas por um modelo de regressão linear e o teste de Tukey foi utilizado para comparação múltipla das médias. A preferência das aves e o respectivo repertório comportamental foram analisados por modelos de regressão mistos, e a idade foi dividida em três categorias: I (aos 9, 12, e 15 d), II (aos 18, 21, 24, e 27 d), e III (aos 30, 33 e 36 d). O efeito de interação entre indicadores ambientais e as semanas foi significativo somente para a iluminância. As aves preferiram o lado NAL a OAL a partir de 18 d (II $P<0{,}001$; III $P=0{,}016$), sendo os comportamentos de beber ($P=0{,}034$) e exploração ou locomoção ($P=0{,}042$) mais frequentes, e a categoria “não visível” ($P<0{,}001$) observada em menor frequência em NAL. Forragear foi o único comportamento que apresentou interação entre a idade e o ambiente, e as aves, durante o período II, expressaram esse comportamento com maior frequência em NAL a OAL ($P=0{,}003$). Em relação às condições experimentais, os frangos de corte preferiram o ambiente NAL a partir de 18 d de idade, quando o efeito de confusão promovido pelas lâmpadas de aquecimento foi removido, e o repertório comportamental também foi diferente de acordo com cada ambiente e idade das aves. Em resumo, as aves indicaram que o fornecimento de luz natural pelas janelas fez diferença relevante em suas vidas, pois foi o ambiente escolhido quando a outra opção dentro do mesmo galpão era de um ambiente com fornecimento exclusivo de luz artificial.

Palavras-chave: ambiente, avicultura, comportamento, luz artificial, luz natural, teste de preferência.

ABSTRACT

Light is an important environmental factor in many aspects for broiler chickens, such as behaviour and physiology, and welfare may be compromised when they are reared under low illuminance. We aimed to investigate what broiler chickens prefer when given free choice between a barn side with artificial lighting only as opposed to the other barn side with natural light through glass windows. Eighty-five 1 d-old male Cobb broiler chickens were divided into 10 pens. The experimental barn was longitudinally divided into a dark side, with no windows and only artificial light (OAL), and the other side was built with a window throughout its lateral wall and received thus both natural and artificial light (NAL); pens were built transversally, so that half of each pen was in the OAL and the other half in the NAL side of the barn. Ten white LED lights were evenly spread across the whole pen area, in both barn sides. The birds chose freely in which side they prefer to staying. Environmental indicators and external conditions such as temperature, relative humidity, air velocity, ammonia concentration and illuminance were monitored inside and outside the experimental barn. Chickens' preference was registered as the number of birds located in each side and their behavioural repertoire, recorded each three days from day 9 to 36 of bird age (totaling 10 d of observation). For the comparison of in-barn OAL and NAL environmental indicators, a linear regression model was fitted, and the Tukey test was used for multiple comparison of means. Bird preference and behaviour data were analyzed using mixed regression models, and age was divided in categories: I (at 9, 12, and 15 d), II (at 18, 21, 24, and 27 d), and III (at 30, 33 and 36 d). The effect of the interaction between environmental indicators and week was statistically different only for illuminance. Chickens preferred NAL to OAL from 18 d onwards (II $P<0.001$; III $P=0.016$). Drinking ($P=0.034$) and exploration or locomotion ($P=0.042$) behaviours were more frequent, and "not visible" behaviours ($P<0.001$) were less frequent, in NAL. Foraging was the only behaviour with an interaction effect between age category and light treatment, as birds during period II expressed this behaviour more frequently in NAL than OAL ($P=0.003$). For our experimental conditions, the chickens preferred NAL from 18 d of age onwards, when the confounding effect of the heating light was removed, and their behavioural repertoire was also different according to each side of the barn and to their ages. In summary, the birds indicated that natural light from windows make a relevant difference in their lives, as it is what they choose when the only other option is the same in-barn environment with only artificial lighting.

Keywords: artificial light, behaviour, environment, natural light, poultry, preference test.

5.1 INTRODUCTION

In general, broiler chickens are intensively reared worldwide in large flocks confined in indoor houses where food, water and environmental control are available to provide for their basic physiological needs (NEWBERRY, 1999). However, considering bird evolutionary history, conditions provided by the production chain are far apart from that found by chickens in a natural life. In nature, they are exposed to a variety of circumstances and environmental conditions which include the day length and photoperiod (COLLIAS; COLLIAS, 1996; NEWBERRY, 1999).

Broiler chickens subjected to commercial management are typically housed in dim lighting because it is presumed improving productivity and feed conversion efficiency, reducing overall activity and injurious pecking (PRESCOTT; WATHES, 1999a; ALVINO; ARCHER; MENCH, 2009). Such inactivity caused by low illuminance is likely related to an apathetic state, as responsiveness to many stimuli seems reduced, even though it is commonly confounded with a calm state (PARANHOS DA COSTA; LIMA; SANT'ANNA, 2017). In fact, light is an important environmental factor for the animals (KRISTENSEN et al., 2007; ALVINO; ARCHER; MENCH, 2009). More specifically for broiler chickens, lighting quality and intensity affect their behaviour and physiology (MANSER, 1996; PRESCOTT; WATHES, 1999a; PRESCOTT; KRISTENSEN; WHATES, 2004; KRISTENSEN et al., 2006; 2007; KUMAR, 2015). Natural lighting as a positive factor for bird welfare is a common assumption. However, it is not clear whether this assumption holds when natural light is offered through glass windows and, thus, in a different constitution as compared to outdoor natural lighting. It remains true, though, that natural lighting through windows may provide a dynamic range of illuminance levels in different areas within the house, with considerably higher intensities as compared to the regular artificial lighting recommended for birds. Thus, the potential for enrichment of the perceived environment and, consequently, for improving bird welfare through barn windows seems to warrant further investigation. The birds do express more natural behaviour and are more active compared to birds not exposed to natural light (BAILIE; BALL; O'CONNELL, 2013). Although there are types of lamps that can offer the same characteristics as natural illuminance, such as bulbs supplemented with ultraviolet (UV) light fixtures (HOUSE et al., 2020), these technologies are not widely used in Brazilian chicken barns, for which a variety of lamp types is observed, such as incandescent and fluorescent lamps (SANS et al., 2021a, 2021b *in press*). In this case, according to the light source type, artificial illuminance may differ from natural light in terms of light colour, intensity, photoperiod and flicker (KRISTENSEN et al., 2006), and these characteristics may influence bird preferences (JAMES et al., 2018). Moreover, worldwide recommendations for illuminance inside the barns accept extremely low levels of 20 lx (DE JONG et al., 2012; RSPCA, 2017) and this seems to represent a major restriction for the animals.

Vision is probably the dominant sense in domestic poultry, and the evolution of vision was determined, in part, by the natural light available (PRESCOTT; WHATES; JARVIS, 2003). The photoreceptive pigments in the retina allow birds to perceive colours in a more detailed way than humans (PRESCOTT; WATHES, 1999b). Birds also have the ability to perceive ultraviolet (UV) light, with the spectral sensitivity below 350 nm (PRESCOTT; WATHES, 1999b; HOUSE et al., 2020), and may experience a better quality of vision in brighter environments (BLATCHFORD et al., 2009). In the natural scenario, UV light is important for birds in relation to orientation, foraging, calibration of their circadian clock and sexual selection (BENNETT; CUTHILL, 1994). In intensive systems, according to glass types, windows may be an alternative for providing some UV wavelengths to chickens (DUARTE et al., 2009; BAILIE; BALL; O'CONNELL, 2013; SILVA; BATISTA; PORFIRO, 2020).

If birds perceive the natural and artificial light in different ways, this may influence their behaviour. Manser (1996) suggested that light intensities between 5 and 22 lx, currently used for broiler chickens and turkeys, may contribute to decrease of their engaging in exploratory behaviour and social interaction, high prevalence of leg abnormalities, mortality, eye abnormalities, breast blisters in growing birds, and fearfulness. Surely, the study of behaviour is an important tool for the identification of relevant environments and devices to the animals, justifying the provision of adequate resources to the animals (FRASER; MATTHEWS, 1997). Preference tests suggest that most broiler chickens make consistent and rational choices associated with the environments that are associated with lower fear and stress responses (NICOL et al., 2009; BROWNE et al., 2010). However, there is a lack of studies about lighting preferences by the birds, and this is especially relevant nowadays, when there is an increase in the number of closed-houses (SOUZA; MOLENTO, 2015). There is an increasing number of companies replacing natural by artificial lighting, in systems that apply the minimal illuminance recommended for broiler chickens houses (20 lx; DE JONG et al., 2012), or even less than the recommended minimum.

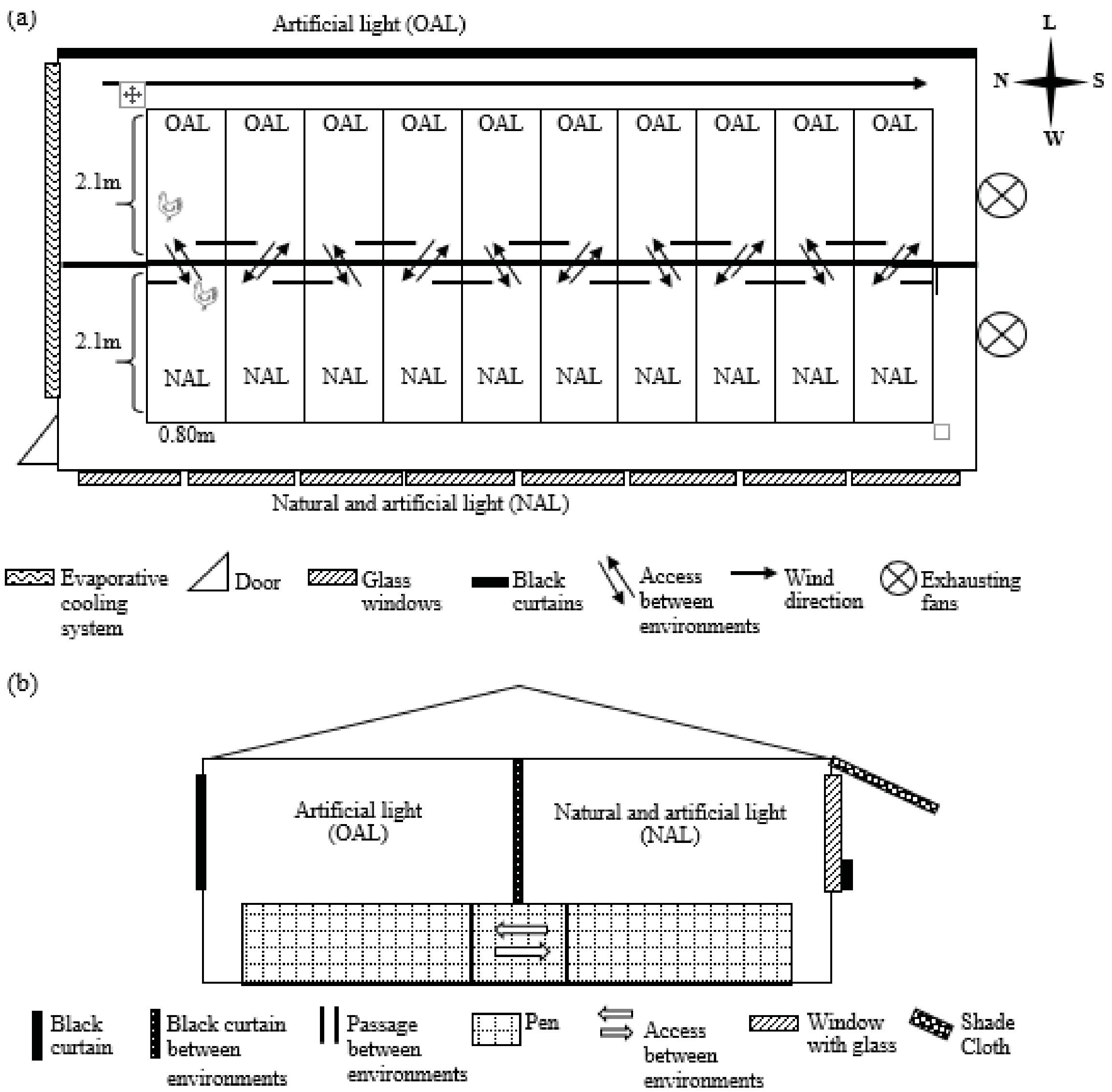
Although there is no public data regarding the proportion of each type of poultry house type in Brazil, basically broiler chickens in intensive systems are raised in two main barn types (SANS et al., 2021a; 2021b *in press*). The conventional system employs open-sided poultry houses, where the natural daylight may enter without passing through a glass when their movable curtains are open; they are called conventional because they used to predominate in the Brazilian poultry meat industry. Lately, the closed-poultry house type is rapidly becoming more popular in Brazil, and it uses only artificial light. Open- and closed-poultry houses have positive and negative welfare aspects, which may also vary according to season (SANS et al., 2021a; 2021b *in press*). However, the quantity and quality of the light available to the birds may be considered a major factor that differentiates these two barn types in terms of their animal welfare potential.

Our objective was to investigate the importance of the existence of windows in the barns, by studying what the chickens prefer when given free choice between an area with only artificial lighting (OAL) and an area with natural and artificial lighting (NAL). Our hypothesis was that the NAL has a significant effect on animal behaviour and that it would be preferred by birds.

5.2 MATERIAL AND METHODS

The study was conducted between January and February 2021, in an experimental broiler house measuring 10 x 6 x 2.5 m (FIGURE 11), of the Federal University of Paraná farm, Pinhais, Brazil (25°23'36.2" S, 49°08'2.9" W) at an altitude of 935 m. The house was built in North-South orientation with 10 pens, each one with a total area of 3.36 m^2 (0.80 x 2.10 m). Eighty-five one-day-old male Cobb broiler chickens were randomly distributed into ten pens, as groups of eight birds in five pens and of nine birds in the other five pens. The experimental design was planned for eight birds per pen and the additional birds were included to cover for eventual mortality throughout the experimental period. The experimental barn was longitudinally divided into a dark side, with no windows and only artificial light (OAL), and the other side was built with a window throughout its lateral wall and received both natural and artificial light (NAL); pens were built transversally, so that half of each pen was in the dark side and the other half in the window side of the barn (FIGURE 12a), resulting in 1.68 m^2 per pen in each barn side. Ten LED lights were evenly spread across the entire pen areas, in both the OAL and NAL sides. The birds were allowed to move freely across the sides as they chose.

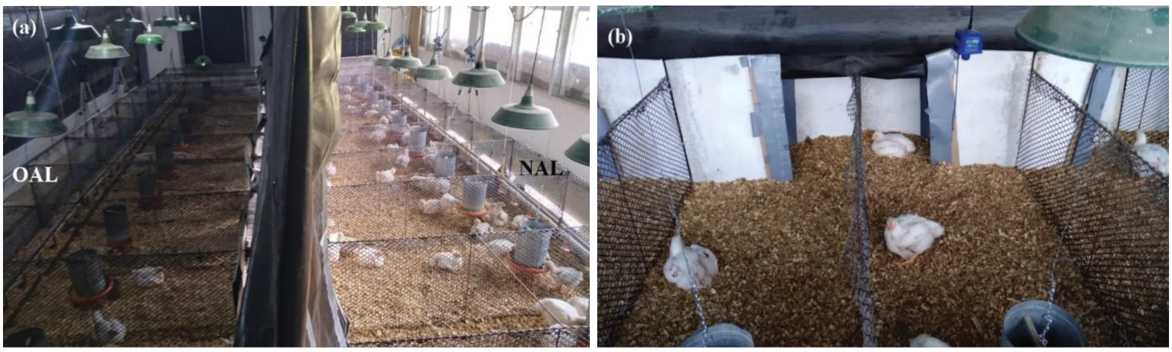
FIGURE 11. EXPERIMENTAL DESIGN OF PREFERENCE TEST SEEN FROM ABOVE (a) AND FROM THE BARN ENTRY SIDE (b). THE HOUSE WAS DIVIDED IN TWO SIDES, ONE WITH ONLY ARTIFICIAL LIGHT (OAL) PROVIDE BY LED LAMPS, AND THE OTHER SIDE, WITH NATURAL LIGHT (NAL) PROVIDED BY GLASS WINDOWS AND ARTIFICIAL LIGHT PROVIDED BY THE SAME LAMP TYPE AND QUANTITY, FROM JANUARY TO FEBRUARY 2021, IN THE STATE OF PARANÁ, SOUTH OF BRAZIL.



Artificial light was provided by Light Emitting Diodes (LED) white lamps of 9 W, 6500 K (correlated colour temperature), DIM, with no UV or infrared emission, distributed along each side of the barn, suspended from the ceiling at a height of 1.50 m from the floor. In NAL sides, in addition to the same quantity and quality of artificial light as in the dark side, natural daylight was provided through eight windows along the west lateral wall of the barn, measuring 1.25 x 0.95 m each, equipped with 8 mm colorless tempered glass. The use of glass, as opposed to the more common open-sided barns in Brazil, was a resource to maintain the control on environmental conditions other than lighting between barn sides, to ensure that birds' preference was based only on illuminance. These windows were partially shut by curtains between 06:00 PM and 07:00 AM by black curtains.

A black curtain was used in the center of the barn to separate the OAL and NAL sides (FIGURE 12a), installed from the ceiling down to 60 cm from the floor. Wooden separators filled this 60 cm close to the floor, and this wooden separation contained passages of 0.50 cm, which allowed for the birds to have free access to both sides of the pen (Figure 12b).

FIGURE 12. OVERVIEW INSIDE THE HOUSE WITH BOTH SIDES (a), OAL (ONLY ARTIFICIAL LIGHT) ON THE LEFT, AND NAL (NATURAL AND ARTIFICIAL LIGHT) ON THE RIGHT SIDE, AND (b) THE SEPARATION BETWEEN EACH SIDE MADE BY BLACK CURTAIN AND WOODEN, IN A PREFERENCE TEST PERFORMED FROM JANUARY TO FEBRUARY 2021, IN THE STATE OF PARANÁ, SOUTH OF BRAZIL.



All the pens were equipped with the same quantity and quality of feed, litter, heaters, manual feeders and drinkers and, from 10 d old onwards, nipple with cups drinkers. Infrared lamps of 240 V, 175 W, for both barn sides and all pens, were used to heat the birds during day and night periods from 1 to 14 d of age, and only during the night period from 15 to 18 d. The heating lamps added, on average, up to 25 lx more in each pen. The pens were made by plastic mesh fence, to facility the air passage. Two exhaust fans, one for each side of the barn, and one evaporative cooling system ensured appropriate temperatures in the entire poultry house. A polyethylene shade cloth was installed on the West side to decrease the direct solar incidence through the glass windows that was observed after 03:00 PM. Natural shadow was provided by trees on the East side of the house (OAL).

5.2.1 Environment measurements

During the experiment, daily and at 10:00 AM and 03:00 PM, outdoor conditions and indoor environmental indicators were measured in the center of each barn side (OAL and NAL), at bird level. Temperature, relative humidity, air velocity and illuminance were measured using Lutron LM 8000A. Ammonia concentration (NH_3) was measured by SP2nd Portable Single-Gas Detector.

5.2.2 Experimental design

On the first day of birds' lives, five groups of birds were initially housed in the OAL side, and the other five groups in the NAL side. Birds had six days of adaptation, for learning between the barn sides offered within each pen, and avoiding any potential confounding effects due to fear of novelty or other factors related to the new environment initially faced by the animals. From day 7 on, each bird group was relocated every three days to the next pen located to their right, allowing for all the groups to stay for three days in each of the 10 pens available in this experiment; this allowed for testing whether there was a pen effect by separating it from group effects. The beginning of assessments started after two days of the group change, allowing the birds to get used to their new pen. In case of mortality, birds were relocated as needed to maintain a minimum of eight birds per pen. Until 18 d, in both sides, the birds received 24L:0D, due the presence of the heating lamps; after this period, the birds received 16L:8D continuous lighting regimen. The lamps were programmed to turn on at 05:30 AM and turn off at 09:30 PM.

5.2.3 Bird preference and behaviour

We video-recorded both sides of two different pens per day, the number of birds in either OAL or NAL sides, and their behavioural repertoire, by fitting four video cameras, Canon Vixia HF R800, one installed in front of each side of each two pens. Recordings started on day 9 and ended on day 36, always from 07:30 AM to 05:30 PM, and were conducted every third day, totaling 10 d of observations with 100 h of video-recordings. The two pens recorded per day were chosen at random, allowed for different pens and groups of birds to be recorded during the experimental period.

Birds' preference was measured by the count of birds present in each side of the barn. Their behaviour was analyzed according to a predefined ethogram (TABLE 17), using the same video-recording. Both count of birds and behaviours were observed by scanning methodology, with instantaneous sampling every 1 h (MARTIN; BATESON, 1993; GUNNARSSON et al., 2008).

TABLE 17. ETHOGRAM WITH DEFINITION OF THE BEHAVIOURS RECORDED FOR BROILER CHICKENS DURING THE PREFERENCE TEST, PERFORMED FROM JANUARY TO FEBRUARY 2021, IN THE STATE OF PARANÁ, SOUTH OF BRAZIL.

Behaviour	Definition
Feeding	Head in the feeder or pecking at the feed within the feeder
Drinking	Beak touching the drinker
Foraging	Pecking or scratching on the floor or both
Exploration or locomotion	Interacting with pen walls or locomotion behaviour, such as running, walking or jumping
Comfort	Preening, wing flapping, wing stretching, feather ruffling or shaking, and elements of dustbathing behaviour
Inactive	Sitting, lying or standing while not engaged in any activity, eyes open or closed
Not visible	Any behaviour that was not identified, due to birds standing very close or in front of each other or in the shielded part of passage ways between barn sides, resulting in an unsatisfactory recording angle

Bird health condition and mortality were checked daily. Birds with severe lameness that compromised their ability to drink and feed, i.e. scores 4 and 5 (WELFARE QUALITY®, 2009), were culled by cervical dislocation.

5.2.4 Statistical analyses

Mortality and outdoor environmental conditions such as temperature, relative humidity, air velocity, NH_3 concentration and illuminance were analyzed by descriptive statistics. For the same environmental indicators, measured indoor and in both barn sides, linear regression models were fitted to test the main effects of house side (OAL or NAL) and age (from 1 to 6 weeks), in addition to the interaction effect. The Tukey's test for multiple comparison was used to ensure a global significance level of 5%, and the goodness of the fitted models was assessed through residual analysis using *half-normal plots* with simulated bands.

Bird preference and behaviour data were analysed by mixed regression models. Total counts of birds in OAL and NAL barn sides, throughout the day for each pen, and the recorded counts were assessed as the response variable. The fixed effect of chicken age and the random effects of group of birds and pens were considered. Age was categorised according to period: I (at 9, 12 and 15 d old), II (at 18, 21, 24 and 27 d old), and III (at 30, 33 and 36 d old). A binomial generalised linear mixed model was initially fitted, but the residual diagnostics clearly pointed that it was inadequate. Then, to account for overdispersion verified in this experimental data, a beta-binomial mixed regression model (NAJERA-ZULOAGA; LEE; AROSTEGUI, 2019) was considered as best adequate fittings.

For each of the remaining behavioural variables, a beta-binomial mixed regression model was also fitted. In such cases, the fixed effects of age categories, side of the barn (OAL or NAL), and the corresponding interaction effect were evaluated. The variables group of birds (birds that were reared together during all experimental period), pen (10 boxes distributed throughout the barn sides) and pen/day (the exact group of birds in each pen for a specific day of behavioural observation) were considered random effects; this last random effect was needed as the design included the rotation of bird groups across pens, thus allowing for the study of any pen effect without the confounding effects of bird group. The fitted models were successively simplified by removing the non-significant fixed effects, starting with the interaction effect, then the main effects of age class and barn side.

The model results were summarised through the estimated probabilities and corresponding confidence intervals (95%). The estimates and standard errors for the variance components of random effects were also presented. The age categories, when statistically significant, were compared using a multiple comparison procedure with properly adjusted P-values.

Statistical analyses were performed using the R software (R CORE TEAM, 2020) and conclusions were based on a significance level of 5%. The contrasts of means for environmental indicators were estimated in the emmeans package (LENTH, 2020). The hnp package (MORAL; HINDE; DEMÉTRIO, 2017) was used for the residual analysis, and the plots were produced through the ggplot2 package (WICKHAM, 2016). The PROreg package (NAJERA-ZULOAGA; LEE; AROSTEGUI, 2020) was used to fit beta-binomial mixed regression models for preference and behaviour analysis.

5.2.5 Ethical approval

This work was approved by the Animal Use Ethics Committee of the Agricultural Campus, N° 104/2017, Federal University of Paraná (ANNEX II).

5.3 RESULTS

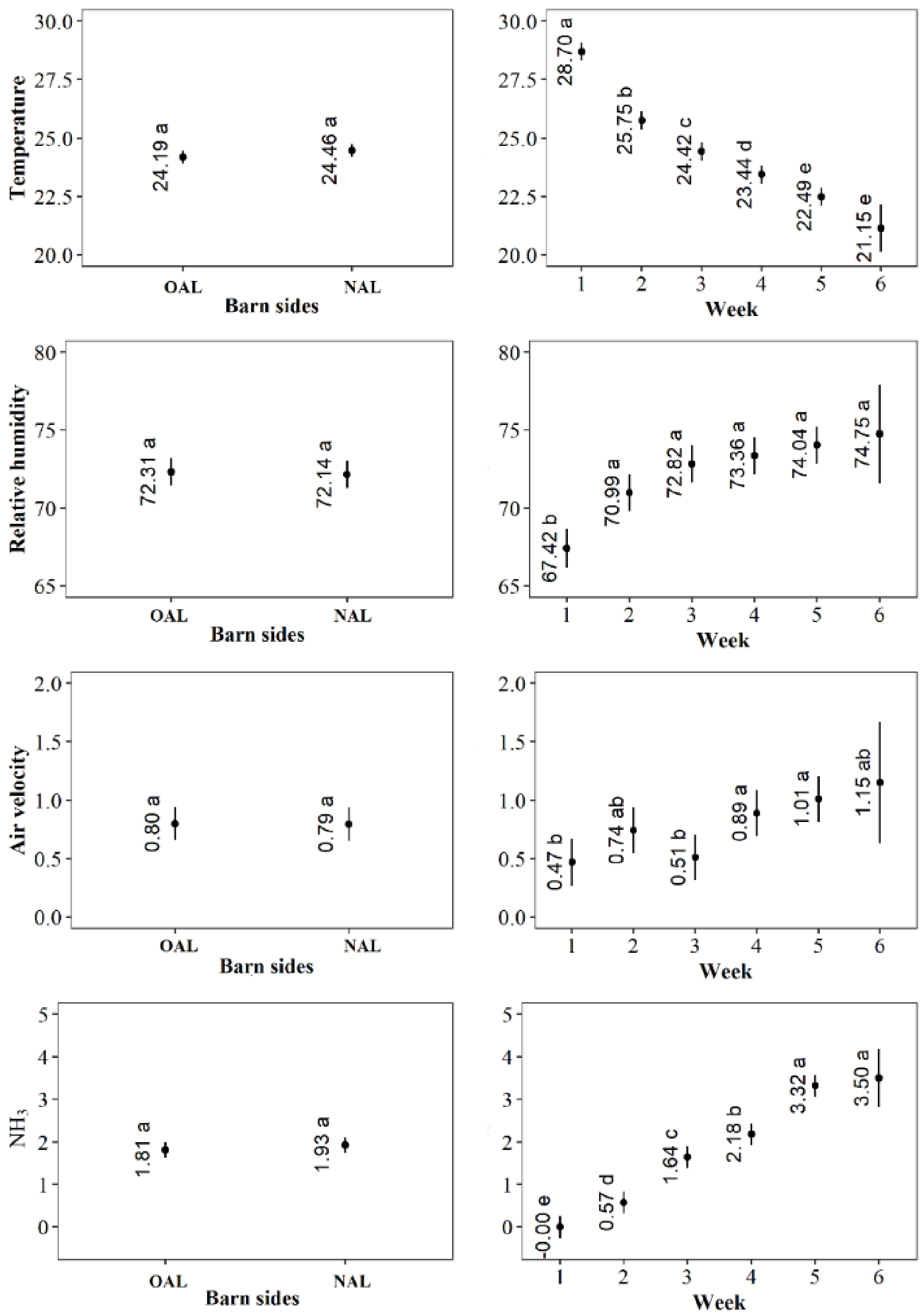
From 2 d, it was observed that some chickens started to move spontaneously between OAL and NAL barn sides, and from 4 d old, at least one bird in each pen had already accessed both sides of the barn. Soon afterwards, from day 6, the number of birds crossing between barn sides became high. Thus, it was not necessary to intercede or teach the birds how to between the barn sides.

The total mortality was 9.4% (8 of 85 birds). The main cause of death, for 4 of the 8 birds, was associated with culling due to severe lameness. Other mortality causes indicated one bird with ascites and another bird with avian infectious bronchitis; the other two birds did not have their deaths investigated, and died at 7 and 13 d.

5.3.1 Environmental measurements

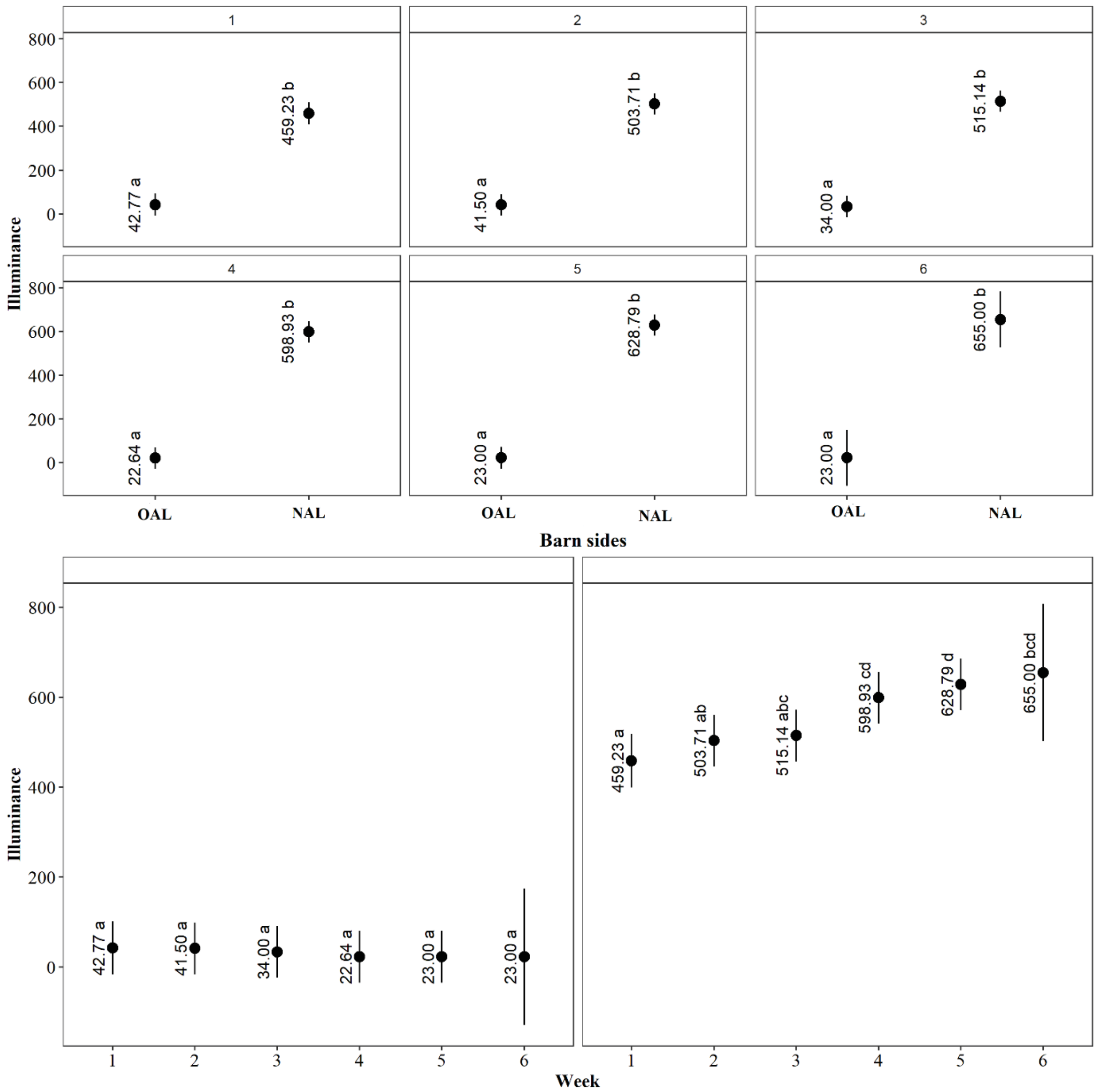
The average (min to max) values for outdoor environmental conditions during data collection periods were: temperature 25.5°C (17.0 to 31.5°C), relative humidity 72.6% (51.0 to 99.9%), air velocity 0.7 m s^{-1} (0.0 to 3.6 m s^{-1}), illuminance 11716 lx (2500 to >20000 lx) and NH_3 concentration 1.0 ppm (0.0 to 2.0 ppm). Results for indoor environmental welfare indicators showed that temperature, relative humidity, air velocity and NH_3 concentration did not differ between OAL and NAL barn sides; however, overall differences across experimental weeks were observed (FIGURE 13).

FIGURE 13. ESTIMATED MEANS AND CONFIDENCE INTERVALS FOR TEMPERATURE (°T), RELATIVE HUMIDITY (%), AIR VELOCITY (M S^{-1}), AND NH_3 CONCENTRATION (AMMONIA; PPM), FOR THE BARN SIDES ONLY ARTIFICIAL LIGHT (OAL), AND NATURAL AND ARTIFICIAL LIGHT (NAL) BARN SIDES, CONSIDERING CHICKEN AGE IN WEEKS, IN A PREFERENCE TEST PERFORMED FROM JANUARY TO FEBRUARY 2021, IN THE STATE OF PARANÁ, SOUTH OF BRAZIL; AVERAGES FOLLOWED BY EQUAL LETTERS DO NOT DIFFER STATISTICALLY (TUKEY TEST, AT 5% SIGNIFICANCE).



Illuminance was the only indoor environmental indicator with a significant effect of the interaction between barn sides and weeks. Even though overall illuminance was significantly higher in the NAL side, it is clear that it significantly increased as weeks went by in the NAL side, while it remained constant throughout the period of six weeks for the OAL side (FIGURE 14). The average (min to max) values for illuminance during all weeks were 32.4 lx (22 to 44 lx) in OAL and 545.5 lx (280 to 900 lx) in NAL.

FIGURE 14. MEANS AND CONFIDENCE INTERVALS FOR ILLUMINANCE (LX), FOR THE ONLY ARTIFICIAL LIGHT (OAL) VS. NATURAL AND ARTIFICIAL LIGHT (NAL) BARN SIDES, CONSIDERING CHICKEN AGE IN WEEKS, IN A PREFERENCE TEST PERFORMED FROM JANUARY TO FEBRUARY 2021, IN THE STATE OF PARANÁ, SOUTH OF BRAZIL; AVERAGES FOLLOWED BY EQUAL LETTERS DO NOT DIFFER STATISTICALLY (TUKEY TEST, AT 5% SIGNIFICANCE).



5.3.2 Bird preference and behaviour

After the heating light was removed, from 18 d of age onwards, results showed in FIGURE 15 suggest that broiler chickens preferred NAL to OAL. This preference was significant for age categories II and III (TABLE 18). Results regarding birds' preference by age categories, not included in the tables, show that birds in period II expressed higher preference for NAL when compared with period III (P=0.007). Averaged for all ages, 32.9% of the birds remained in OAL while 67.1% in NAL.

FIGURE 15. PERCENTAGE OF BROILER CHICKENS OBSERVED IN THE NATURAL AND ARTIFIAL LIGHT (NAL) BARN SIDE, ACCORDING TO BIRD AGE CATEGORY (I AT 9, 12, 15 D OLD; II AT 18, 21, 24, 27 D OLD; III AT 30, 33, 36 D OLD), IN A PREFERENCE TEST PERFORMED FROM JANUARY TO FEBRUARY 2021, IN THE STATE OF PARANÁ, SOUTH OF BRAZIL; DIFFERENT LETTERS REFER TO SIGNIFICANT DIFFERENCES AMONGST BIRD AGE CATEGORIES, AND DASHED LINE IS THE GRAPHIC REPRESENTATION OF THE POINT OF BARN SIDE PREFERENCE.

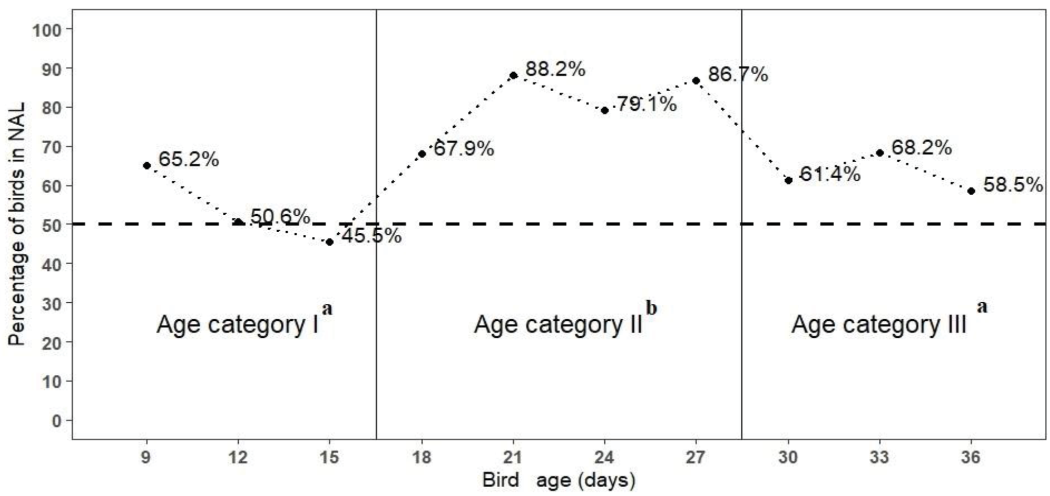


TABLE 18. ESTIMATED PREFERENCE PROBABILITIES, FOR THE NATURAL AND ARTIFICIAL LIGHT (NAL) BARN SIDE, ACCORDING TO BIRD AGE CATEGORY, IN A PREFERENCE TEST PERFORMED FROM JANUARY TO FEBRUARY 2021, IN THE STATE OF PARANÁ, SOUTH OF BRAZIL.

Bird age category		Preference		
Period	Observation days	Estimates[1]	CI (95%)	P-value[2]
I	9, 12, 15	0.538^a	(0.435 ; 0.637)	0.470
II	18, 21, 24, 27	0.803^b	(0.724 ; 0.864)	<0.001
III	30, 33, 36	0.627^a	(0.523 ; 0.719)	0.016

$\hat{\sigma}^2$group=0.169 (0.096) $\hat{\sigma}^2$pen=0.191 (0.090)

[1] Different letters means different probabilities (α=5%)

[2] P-value for testing the null hypothesis of random barn sides choice

Results regarding feeding and comfort behaviours showed no window effect (TABLE 19), but a significant effect of the age categories. The difference in frequency of feeding behaviour was significant between period I *vs* period III (P=0.020). The frequencies for comfort behaviour were different across all the three age categories: period I *vs* period II (P=0.002), period I *vs* period III (P<0.001) and period III *vs* period II (P=0.036). The presence of the window was a significant factor for drinking (P=0.034) and exploration or locomotion behaviours (P=0.042), which were more frequent in NAL. The category "not visible" showed higher counts in OAL (P<0.001), and the only behaviour observed was "any behavior that was not identified, due to birds standing in the shielded part of passage ways between barn sides due to unsatisfactory recording angle". There was no significant effect for inactive behaviour (P>0.05) and this was the most common behaviour in both OAL (47.0%) and NAL (44.6%) barn sides.

TABLE 19. ESTIMATED BEHAVIOUR PROBABILITIES, ACCORDING TO THE PRESENCE OF WINDOWS (OAL VS NAL) AND BROILER CHICKEN AGE CATEGORY (PERIOD I, II, III), IN A PREFERENCE TEST PERFORMED FROM JANUARY TO FEBRUARY 2021, IN THE STATE OF PARANÁ, SOUTH OF BRAZIL.

Behaviour	Effect				
	Bird age category[1]		Window[1,2]	Estimates[3]	CI (95%)
	Period	Observation days			
Feeding	I	9, 12, 15	ns	0.343^{a}	(0.279 - 0.413)
	II	18, 21, 24, 27		0.275^{ab}	(0.219 - 0.337)
	III	30, 33, 36		0.217^{b}	(0.163 - 0.281)
$\hat{\sigma}^2$group=0.065 (0.091)		$\hat{\sigma}^2$pen=0.073 (0.083)		$\hat{\sigma}^2$pen/day=0.113 (0.070)	
Comfort	I	9, 12, 15	ns	0.034^{a}	(0.023 - 0.052)
	II	18, 21, 24, 27		0.086^{b}	(0.068 - 0.109)
	III	30, 33, 36		0.123^{c}	(0.097 - 0.156)
$\hat{\sigma}^2$group=0.089 (0.077)		$\hat{\sigma}^2$pen=0.367 (0.098)		$\hat{\sigma}^2$pen/day=0.057 (0.067)	
Drinking	ns		OAL	0.026^{a}	(0.016 - 0.041)
			NAL	0.045^{b}	(0.035 - 0.059)
$\hat{\sigma}^2$group=0.208 (0.142)		$\hat{\sigma}^2$pen=0.379 (0.146)		$\hat{\sigma}^2$pen/day=0.535 (0.125)	
Exploration or locomotion	ns		OAL	0.031^{a}	(0.020 - 0.049)
			NAL	0.053^{b}	(0.042 - 0.068)
$\hat{\sigma}^2$group=0.493 (0.158)		$\hat{\sigma}^2$pen=0.083 (0.099)		$\hat{\sigma}^2$pen/day=0.191 (0.083)	
Not visible	ns		OAL	0.118^{a}	(0.088 - 0.156)
			NAL	0.035^{b}	(0.024 - 0.053)
$\hat{\sigma}^2$group=0.174 (0.125)		$\hat{\sigma}^2$pen=0.327 (0.127)		$\hat{\sigma}^2$pen/day=0.440 (0.110)	
Inactive	ns		ns	0.455	(0.416 - 0.494)
$\hat{\sigma}^2$group=0.053 (0.069)		$\hat{\sigma}^2$pen=0.086 (0.059)		$\hat{\sigma}^2$pen/day=0.122 (0.055)	

[1] ns = no significant
[2] OAL = only artificial light; NAL = natural and artificial light
[3] Different letters means different probabilities (α=5%)

There was a significant effect for the interaction between windows and age categories for foraging behaviour (TABLE 20): when chickens were younger, in period I, they foraged more frequently in NAL than OAL (P=0.003), while for the other two age categories, there was no difference. Considering the behaviour observed when the chickens were on the NAL side, birds in period I foraged more frequently than when they were in age category II (P<0.001); the difference remained significant when birds in period I were compared with the same birds in period III (P=0.009). There were no differences across the age categories when the chickens were observed in the OAL barn side.

TABLE 20. ESTIMATED PROBABILITIES FOR FORAGING BEHAVIOUR, ACCORDING TO THE PRESENCE OF WINDOWS (OAL VS NAL) AND BROILER CHICKEN AGE CATEGORY, IN A PREFERENCE TEST PERFORMED FROM JANUARY TO FEBRUARY 2021, IN PARANÁ STATE, SOUTH OF BRAZIL.

Bird age category		Window presence	
Period	Observation days	OAL[1,2]	NAL[1,2]
I	9, 12, 15	0.014^{Aa} (0.005; 0.037)	0.067^{Ba} (0.045; 0.097)
II	18, 21, 24, 27	0.009^{Aa} (0.002; 0.036)	0.007^{Ab} (0.004; 0.016)
III	30, 33, 36	0.019^{Aa} (0.006; 0.058)	0.009^{Ab} (0.003; 0.027)

$\hat{\sigma}^2$group=0.343 (0.242) $\hat{\sigma}^2$pen=0.853 (0.271) $\hat{\sigma}^2$pen/day=0.453 (0.207)

[1] Different capital letters refer to significant differences between barn sides, and different low case letters indicate significant differences between birds' age
[2] OAL = only artificial light; NAL = natural and artificial light

5.4 DISCUSSION

In general, our results showed that, after the heating light was removed, from 18 d of age onwards, broiler chickens preferred NAL to OAL. This preference was significant for age categories II and III. The chickens spent more time drinking, explorating and moving, and foraging in NAL than OAL. Inactive (the most commonly observed behaviour), feeding and comfort behaviour did not differ significantly between OAL vs NAL, only according to bird age category.

Regarding birds' preference, our results are in agreement with other studies which showed that birds chose environments with higher illuminance and also expressed other changes in their behavioural repertoire due to differences in light intensity (DAVIS et al., 1999; PRESCOTT; WHATES, 2002; RAULT et al., 2017; RACCOURSIER et al., 2019), and in our study we observed average of 32.4 lx in OAL and 545.5 lx in NAL. According to Lima, Silva (2019), the absence of natural light, especially in closed-sided houses, may limit the expression of natural behaviours, with negative impacts on chicken welfare. Prescott, Kristensen, Whates (2004) strongly recommend a combination of natural daylight and artificial light for poultry barns. These considerations regarding the use of natural light are dependent on the importance of this choice for the birds themselves, with a potential to improve their welfare which tends to be proportional to the importance of natural light from the point of view of the birds. Our results especially contribute to the understanding of the birds preference, as the only internal environmental indicator that showed significant difference between OAL and NAL barn sides was illuminance. This represents an overall response of the birds to light conditions which warrants further studies, to understand the importance of other light characteristics, such as wavelength or spectrum variances. The light intensity is one of the most studied light characteristics for broiler chickens (DAVIS et al., 1999; BLATCHFORD et al., 2009; RAULT et al., 2017; RACCOURSIER et al., 2019), and the bird preference for higher illuminance encouraged behaviours such as drinking, exploration or locomotion and foraging in our study.

Solar radiation reaching the earth surface is divided into infrared radiation, visible light, and UV; the latter is divided into three types according to wavelength: UVA (315-400 nm), UVB (280-315 nm), and UVC (100-280 nm), but 99% of the UV that reaches earth is UVA (LEWIS; GOUS, 2009). The solar radiation types that effectively reach individuals vary according to existence and type of eventual physical barriers. Tempered glass, 4 mm, may block up to 28.4% of UV light from reaching the individuals (DUARTE et al., 2009), and 8 mm, 54.5% (SILVA, BATISTA, PORFIRO, 2020). The glass type may also blocked at least 90% of wavelengths under 350 nm (DUARTE et al., 2009; LEWIS; GOUS, 2009). However, windows with glass allow both visible wavelengths and a small amount of UV to pass to inside the houses (BAILIE; BALL; O'CONNELL, 2013) and, thus, alter chicken behaviour (DE JONG; GUNNINK, 2018). In the NAL barn side, birds may have received UV light that was not available in OAL side. This may have motivated their preference, as poultry have a fourth retinal cone photoreceptor that allows them to see in the UVA wavelength (315-400 nm;

PRESCOTT; WATHES, 1999b). Birds exposed to some UV light may have decreased stress susceptibility and fear responses than those raised without UV (HOUSE et al., 2020), showing that the illumination of poultry houses can be improved in several aspects.

Regarding lamp types, LED bulbs with colour temperatures over 5000 K, called cold (ARCHER, 2018), contain more blue than warm white light (SULTANA et al., 2013), and in our study 6500 K lamps were used. Thus, when birds preferred to stay in the NAL barn side, in addition to high light intensity they may have also chosen it because 6500 K is an average natural daylight colour temperature (PRESCOTT; WHATES; JARVIS, 2003; KRISTENSEN et al., 2006). Thus, in our study, the OAL light source was similar specifically in terms of colour temperature to the average daylight that birds were searching for by moving to the NAL side of the barn, suggesting that in this sense the intensity may have been the main driver for the preference.

New lighting technologies are currently being developed as potential replacements for incandescent light sources, and some sources may be better to the welfare of broilers chickens (OLANREWAJU et al., 2016). However, our results suggest that the exposure to natural lighting may be an ideal solution according to the preference of the birds. This warrants further preference studies with different types of artificial light bulbs, as well as asking the birds how strong their preference is, through motivation tests. Considering the higher visual perception capacities that birds have as compared to humans, it seems relevant to explore light characteristics additional to intensity to better understand what the birds are responding to when they express their preferences. A photoperiod of 16L:8D also appears to maximise chicken welfare, as their behaviour patterns tend to become more synchronised when there is pronounced intensity contrast between the light and dark period (NICOL et al., 2017). This contrast was more evident in the NAL barn side, as the windows allow for some contact of the animals with the natural dawn and dusk periods. In future research, the real perception of birds in relation to illuminance may be further studied. Although the differences in perceived light intensity by birds, known as Clux or Gallilux, may be estimated by adding between 20-25% in relation to lux, i.e. 25 Clux = 17.4 lx (LEWIS; MORRIS, 2000; OLANREWAJU et al., 2018; 2019), it is important to study light from a bird perspective, with more precision technology.

Bird preferences may be influenced not only by barn sides and their characteristics regarding light, but by their natural behaviours (FRASER; MATTHEWS, 1997). A special consideration is that chickens are social animals, and bird preferences may be influenced not only by individual choices, but also by their social nature and its effects, such as social facilitation (PRESCOTT; KRISTENSEN; WHATES, 2004; BESSEI, 2006; GUNNARSSON et al., 2008). Because of social facilitation, the birds tend to behave as a social unit, where most members exhibit the same behaviour at the same point in time (PARANHOS DA COSTA; LIMA; SANT'ANNA, 2017). Thus, the higher number of chickens in NAL side may have acted as an additional force for more birds to migrate to this side.

Bateson, Seanurne-Way (1973) suggested that when birds were exposed to constant light, the elicitation of social behaviour becomes more likely. Our results seem to reinforce the statement that a place with higher illuminance fosters group formation that may be positive for the animals. Recognition between individuals is also part of the social interaction process, and this characteristic may be affected when birds are reared in very low illuminance (MANSER, 1996; PRESCOTT; WATHES, 1999a; PRESCOTT; WHATES; JARVIS, 2003). According to Porter et al. (2005), chicks that had been housed in pairs in the dark showed no evidence that they discriminated between familiar and unfamiliar test partners. Thus, the NAL side may also have provided a better recognition of individual birds and, consequently, this may be potentially considered an additional factor explaining bird choice. Collins et al. (2011) reinforce the importance of vision in key behaviours such as feeding and social behaviour in poultry, and suggest that the birds may experience lower welfare as a result of their lack of sight. Therefore, when birds choose the NAL barn side, they may be making choices to favour their natural social interaction behaviours.

The birds also spent a considerable proportion of their time in OAL barn side, and this choice should also be considered. Ideally, birds should have access to different types of illuminance, so that they can choose according to their preferences. Accordingly, the provision of areas with reduced light intensity for resting and other activities has been suggested before (RACCOURSIER et al., 2019). On farms where windows are provided, resting behaviour occurs more often in areas with lower light intensities, whereas active behaviours occur in areas with higher light intensities, but it is up to the birds to choose (SOUZA DA SILVA; DE JONG, 2019). This pattern of light intensity choices is expected for diurnal animal species. Vergneau-Grosset, Peron (2020) recommended that when exposing an animal to UV light, it is important to provide a hiding place or shade; in our study, both the passage way between each barn side and the OAL side may have fulfilled this function. Negative effects of excess UV radiation intensity may be observed in both natural and artificial light sources, and revolve around the occurrence of burns in animals and behavioral changes, such as increased stress or incidence of severe feather pecking (RUIS et al., 2010; VERGNEAU-GROSSET; PERON, 2020). During the experimental period, none of these characteristics were observed in our birds, which agrees with the probably low UV exposure through the glass window.

In our study, the birds showed preference for the NAL barn side only from 18 d onwards. The association of this preference with bird age was also observed during other preference test, when chicks spent most time in the brightest light (200 lx) at 2 weeks, and at 6 week the birds preferred the environment with dimmest light (6 lx; DAVIS et al., 1999). The age for birds to begin expressing light preferences coherently coincides with the total removal of the heating lamps. Although this type of lamp is not suitable for lighting, it was responsible for adding up to 25 lx in each pen, which may have acted as an important confounding effect for birds to detect the lighting differences between barn sides. In addition, according to Gunnarsson et al. (2008), early exposure to natural or artificial light might have an effect on later preference for light type and on the behaviour of the birds, even after a house transition. Therefore, the birds may have grown habituated with the illuminance from the heating lamps and, after their removal, they may have been obliged to make new choices, as their early life light experience became absent. In addition, the heating lamps may have provided early imprinted association between light intensity and heat, reinforcing a positive perception of light by the birds. Even though it was not possible to identify the exact reason for bird preference for the NAL barn side, most possible explanations seem coherent with the more natural characteristic of the lighting on this side of the barn. Our hypothesis is that the windows tend to be closer to meeting the birds’ basic needs in relation to light and, thus, tend to increase animal welfare. Examples of such needs include the establishment and maintenance of social hierarchies, social encounters, group aggregation and peer recognition.

Results regarding chicken behaviours showed that the frequencies varied according to barn sides (drinking, exploration and locomotion, and not visible), and bird age categories (feeding and comfort). The behaviours of drinking, exploration and locomotion showed higher frequencies in the NAL side, and the category not visible birds was more frequent in the OAL side. Davis et al. (1999) observed that broiler chicks performed more feeding, drinking and locomotion behaviours in the brighter environments. However, for Deep et al. (2012) light intensity had no effect on expression of drinking behaviour. Adding further evidence to this discussion, our results indicate that providing windows increases the behaviour repertoire, a fact observed in previous studies. Sans et al. (2021a; 2021b *in press*) observed that broiler chickens reared in open-sided houses, with natural light provided by no-glass windows, but with curtains during summer/autumn showed higher relative frequencies for exploration behaviour, when compared with birds in closed-sided houses; during the winter, there was a higher frequency for drinking and a lower inactivity. Thus, our results suggest that, even with eventual changes in natural daylight characteristics due presence of glass in the windows, it remains possible to observe a potential improve in bird welfare as the increase of activities considered important for the birds, i.e. social activities in the NAL barn side. Furthermore, windowed industry barns in Brazil do not fit glass barriers, this was an experimental resource to control for other in-barn environmental conditions such as temperature and relative humidity, in order to study the specific effect of lighting. These results reinforce that, when given the opportunity, birds prefer to perform their behaviours in an environment with natural daylight or, minimally, higher levels of illuminance than those provided inside the barns with only artificial lighting.

The exploratory or locomotion behaviours, observed in higher frequency in NAL side, tend to be viewed as positive interactions between the birds and their environment (NEWBERRY, 1999). However, if the house is not stimulating, as birds age they may get bored and reduce exploratory behaviour (NEWBERRY, 1999). It seems important that broiler chickens are reared in stimulating poultry houses, with adequate lighting characteristics that allow for the birds to perform activities which are essential for their welfare.

Foraging was the only behaviour for which a significant interaction effect between window and age categories was present, indicating that birds foraged more, when were younger, in NAL than OAL barn side. Alvino et al. (2009) also observed that foraging was affected by light intensity, and broiler chickens in the 5 lx treatment spent significantly less time performing this behaviour than when the light intensity was 50 and 200 lx. Foraging, exploration or locomotion are important behaviours, since they involve actions related to knowing the environment and searching for feed. According to Manser (1996), newly hatched birds, both domestic poultry and turkeys, may die of malnutrition if they have difficulty in seeing the feeders due low light intensity, which may reduce overall activity, reducing the chances of foraging, finding a feeder and learning how to feed. Although this describes an extreme situation, it demonstrates the importance of adequate lighting from the first days of birds' life, so that they can enjoy the opportunity to explore the environment, the other birds as well as the resources available.

Inactive behaviour was not different between barn sides or across different age categories. According to some studies, this behaviour may be associated with increased bird age, walking ability deteriorated, body weight and fast growth rates (WEEKS et al., 2000; BESSEI, 2006; BAILIE; BALL; O'CONNELL, 2013). Although our study did not test the birds' walking ability, the higher number of culls regarding leg problems suggests that this problem was prevalent, causing suffering and pain to the birds, as well as limiting their behavioral repertoire.

Although light is an important element for birds, when provided in isolation, it may not be enough to reduce inactive behaviour. According to El-Deek, El-Sabrout (2019), most of intensive production systems that are currently used do not usually support the natural behavioural needs of poultry. Therefore, farm animals may be reared in an environmental with enrichment and light which more closely resembles their natural characteristics. These options, acting together, may increase activity, improve leg health (SHERWIN; LEWIS; PERRY, 1999; BAILIE; BALL; O'CONNELL, 2013) and stimulate behaviours such as foraging and exploration (DE JONG; GUNNINK, 2018). However, selection for fast growth may lead to several welfare problems, such as metabolic disorders, decrease locomotor activity and extend time spent sitting or lying (BESSEI, 2006). For EFSA (2010), the risk assessment regarding poor welfare effects showed that fast growth is one of the major risk scores, including unbalanced body conformation, high stocking density, wet litter, and light intensity. Include slower-growing genetic strains may be a way to decrease welfare restrictions

(BESSEI, 2006; HARTCHER; LUM, 2019), added to important environmental changes indoor houses to meet the birds' needs in the current poultry industry.

In general, animals engaged in pleasant activities, such as exploring, feeding and interacting with other animals in a social group, may experience positive feelings, and without this engagement, the animal will not experience the full range of positive welfare states that are potentially available (MELLOR, 2014). Although in our study, a qualitative behavioral assessment (WELFARE QUALITY, 2009) was not used, it is likely that birds, were more likely to experience positive feelings while they were in the NAL barn side, due to higher opportunities to explore, forage, move and interact with other birds.

Some behaviours were only also associated with age, such as comfort and feeding. Comfort behaviours were associated with increases in bird age categories. In the literature, this behaviour is associated to increases in chicken welfare, as the activities may related to the maintenance of bird health (APPLEBY; MENCH; HUGHES, 2004). Alvino et al. (2009), observed that broiler chickens reared in 5 lx spent less time in preening behaviour, as compared to those in 50 and 200 lx. The increase of comfort may be understood as positive results, indicating a possibility to encourage higher expression of behaviours associated to increases in chicken welfare. Although we only observed difference in feeding frequency regarding bird age categories, some authors observed a clear preference of laying hens and broiler chickens to eat in brighter lightings, from 20 to 200 lx (PRESCOTT; WATHES, 2002; RACCOURSIER et al., 2019), and that they ate more under 30 lx than 1 lx (LI et al., 2020). Birds may also find it aversive to eat in very dim light, because this behaviour is normally guided visually, and they see better in brighter environments (PRESCOTT; WATHES, 2002; BLATCHFORD et al., 2009). Although feeding behaviour decreased with age in our study, no emaciated chicken was observed during the experimental period and feeding showed the second highest frequency, only behind inactive behaviour.

As for the "not visible" behavioural category, when the birds were younger, some of them stayed together in the passage between the barn sides, which may have given an enhanced sense of social interaction or protection. As birds aged, they may also have been looking for a different lighting, according to specific momentaneous needs. Birds observed in OAL spent less time exploration, moving and foraging, and when observed in this barn side, stayed lying very close or in front of each other, which also prevented appropriate behavioural identification. Thus, a potential reason for finding more birds in the not visible behaviour category in the OAL side may be an association between seeking an environment with lower light intensities and pen areas associated with a feeling of protection, provided by staying either close to wall angles in the passageways or close to another bird. Such potential reason seems to indicate that the OAL side was chosen by the birds when they were searching for a cozy place to either rest or sleep.

5.5 CONCLUSION

For our experimental conditions, the chickens preferred natural and artificial lighting from 18 d of age onwards, when the confounding effect of the heating light was removed, and their behavioural repertoire was also different according to each side of the barn and to their ages. As the chickens also used the lower lit pen areas, barns with light gradient options seem important for them. In summary, the birds indicated that windows make a relevant difference in their indoor lives, as it is what they choose when the only other option is the same in-barn environment with only artificial lighting.

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6. FINAL CONSIDERATIONS

The number of broiler chickens involved in the production systems is high and these birds may be exposed to several situations that may decrease their welfare. Inadequate environmental conditions, such as high densities, low illuminance, rapid growth that can affect their locomotor capacity, etc., which directly affect the welfare of each animal. The contemporary animal welfare science is composed of five domains, nutrition, environment, health, behavior, and mental state, and all these components must run aligned in a responsible and ethical animal production system. As such, the sector has a key role for strengthening animal welfare in the current poultry industry.

This thesis contributed to verify that, although there are different poultry houses adopted in South of Brazil, two house types may be highlighted, and both present challenges to birds, regarding environment, health, behavioral and mental aspects.

During summer/autumn seasons, it was possible to identify that closed-sided houses were related to worse welfare results considering three environmental indicators (light intensity, NH_3 and CO_2 concentrations) and two animal-based indicators (contact dermatitis on the breast and abdominal areas and exploratory behaviour), while open-sided houses showed worse results for one environmental indicator (air velocity), and two animal-based indicators (scratches and panting behavior). However, this scenario changes according to seasons. During winter season, in closed-sided houses there were better results for two environmental indicators (air velocity and NH_3 concentration) and four animal-based indicators (contact dermatitis on the breast and abdominal areas, bird soiling, hock burns, and touch test), while in open-sided barns there were fewer behavioural restrictions and more positive emotional states (animal-based indicators). Overall, in both houses low welfare conditions prevailed, as visually demonstrated in TABLES 21, 22 and 23.

TABLE 21. AVERAGE OF ENVIRONMENTAL INDICATORS CLASSIFIED ACCORDING TO BROILER CHICKEN WELFARE SCALE, ASSESSED IN CLOSED- AND OPEN-SIDED HOUSES, DURING SUMMER/AUTUMN AND WINTER SEASONS 2019, IN THE WEST OF SANTA CATARINA STATE, SOUTH OF BRAZIL§

Environmental indicators	Season / House designs			
	Summer/Autumn		Winter	
	Closed-sided	Open-sided	Closed-sided	Open-sided
Stocking density (birds/m²)[1]	13.9 ± 0.4*	12.0 ± 0.3*	13.8 ± 0.2*	12.0 ± 0.2*
NH_3 (ppm)[2]	11.2 ± 6.8*	7.5 ± 3.9*	12.5 ± 14.4*	15.3 ± 11.7*
CO_2 (ppm)[2]	1124.9 ± 461.5*	841.0 ± 158.0*	2047.1 ± 994.8	
Illuminance (lx)[2,3]	6.9 ± 6.3*	274.2 ± 241.9*	18.6 ± 14.5*	353.5 ± 478.1*
Relative humidity (%)[2,4]	73.5 ± 12.3		76.7 ± 13.7*	72.1 ± 12.7*
Temperature (ºC)[2,4]	25.9 ± 2.0	23.4 ± 3.0*	21.5 ± 2.7*	
Air velocity (m s^{-1})[5]	2.1 ± 0.7*	1.1 ± 1.0*	2.5 ± 1.9*	0.1 ± 0.4*

§ Color code used: EXCELLENT | ENHANCED | ACCEPTABLE | POOR | VERY POOR

* Results with significant difference between house designs (P<0.05).
Comparison with results from other studies for the adoption of the animal welfare scale: [1] WAP, 2019; [2] EFSA, 2012; DEFRA, 2018; [3] PRESCOTT et al., 2003; [4] FURLAN; MACARI, 2002; [5] COBB, 2012

TABLE 22. AVERAGE OF HEALTH INDICATORS ASSESSED ON FARM AND AT THE SLAUGHTERHOUSE, CLASSIFIED ACCORDING TO BROILER CHICKEN WELFARE SCALE, ASSESSED IN CLOSED- AND OPEN-SIDED HOUSES, DURING SUMMER/AUTUMN AND WINTER SEASONS 2019, IN THE WEST OF SANTA CATARINA STATE, SOUTH OF BRAZIL§

Health indicators	Season / House designs			
	Summer/Autumn		Winter	
	Closed-sided	Open-sided	Closed-sided	Open-sided
Contact dermatitis on the breast and abdominal areas (%, 2+3) [1]	8.2*	4.4*	5.9*	22.9*
Bird soiling (%, 2+3) [1]	11.7		5.4*	24.5*
Hock burn (%, 2+3+4) [1,2]	1.5		1.2*	9.3*
Footpad dermatitis (%, 2+3+4) [1,2,3]	24.6		42.3	
Lameness (%, 3+4+5) [2,3]	42.7		60.4	
Scratches (%, 1+2+3) [1]	59.6*	66.8*	77.4	

§ Color code used: EXCELLENT | ENHANCED | ACCEPTABLE | POOR | VERY POOR

* Results with significant difference between house designs (P<0.05)
Comparison with results from other studies for the adoption of the animal welfare scale:[1] SOUZA et al., 2018; [2]SOUZA et al., 2015b; [3] SANS et al., 2014; FEDERICI et al., 2016

TABLE 23. AVERAGE OF BEHAVIOURAL AND BIRD AFFECTIVE STATES, CLASSIFIED ACCORDING TO BROILER CHICKEN WELFARE SCALE ASSESSED IN CLOSED- AND OPEN-SIDED HOUSES, DURING SUMMER/AUTUMN AND WINTER SEASONS 2019, IN THE WEST OF SANTA CATARINA STATE, SOUTH OF BRAZIL§

Behaviour and bird affective states	Season / House designs			
	Summer/Autumn		Winter	
	Closed-sided	Open-sided	Closed-sided	Open-sided
Panting (%) [1]	9.0*	16.2*	3.2	
Exploration (%) [2]	1.2*	1.8*	1.3	
Drinking (%) [3]	6.3		8.0*	11.0*
Inactive (%) [4]	53.3		65.0*	57.2*
Touch test (0-100 score) [1,5]	88.2		98*	67*
Qualitative behaviour assessment [5]	Prevalence of negative emotional states		Prevalence of negative emotional states*	Prevalence of positive emotional states*

§ Color code used: EXCELLENT | ENHANCED | ACCEPTABLE | POOR | VERY POOR

* Results with significant difference between house designs (P<0.05).
Comparison with results from other studies for the adoption of the animal welfare scale: [1] TUYTTENS et al., 2015; [2] SCHÜTZ, FORKMAN, JENSEN 2001; [3] SANS et al., 2014; [4] BACH et al., 2019; [5] FEDERICI et al., 2016;

Furthermore, our research added knowledge about specific areas inside each house type that may decrease animal welfare, with worse welfare problems in the West direction, which in closed-sided means near the exhausting fans and in open-sided, the direction of positive-pressure mechanical ventilation by fans.

Regarding the preference test, we observed that the birds choose an environment with higher illuminance, resulting in differences in their behaviour repertoire. This choice is influenced by bird age, which is a result that seems associated to the use of heating lamps in the first two weeks of age. Thus, there is a gap concerning the recognition of bird cognition, their capacity to experience and express preferences and suffering, and what is really offered to the animals. There are opportunities to offer conditions for the chickens to live positive experiences, which are directly linked to their welfare, which deserve consideration during decision-making. Discussions about poultry houses that may offer greater opportunities for birds to choose according to their preferences throughout both daytime and lifetime, such as different illuminances or options regarding environmental enrichment, seem relevant and may contribute to improve the ethics of the industrial system.

In this thesis, each chapter indicates points to be improved. However, in general, each barn only partially attends to the needs of the birds, and even within each barn type, some areas may compromise the welfare of the birds in a more or less severe way and may vary according to the season. We hope that the results from this study may be used by professionals of the poultry industry, contributing to empowering better decisions and action plans for the adoption of strategies that spread best conditions throughout the internal barn area in both closed- and open-sided house designs. In addition, our work also emphasizes the relevance of constant bird welfare monitoring, especially in key locations within the barns.

Technology is important and may be extremely useful to meet the animal needs, allowing for viability, efficiency and cost reduction to the monitoring of animal welfare. However, the relationship between human and non-human animals remains fundamental for any process to be based on ethics and respect. The daily observation of birds, their behavior and preferences, investment in training that are based on the pillars of animal welfare, and the appreciation of people who work with the animals are all part of required improvements. It is a fact that the focus of animal production is productivity, and conflicts emerge from this fact. Thus, much broader changes are needed in the way food is produced. Meanwhile, it is essential to remember that within each chicken barn, there are thousands of sentient individuals, under our responsibility and that are dependent on our attitudes.

Changes in the industrial systems and respective house types are necessary; however, such changes must be conceived considering the interests of the animals. Brazil is one of the main countries involved in chicken production; therefore, it may also become a protagonist in issues related to chicken welfare and meet the global demands of society concerning more production systems that are more considerate in terms of animal welfare.

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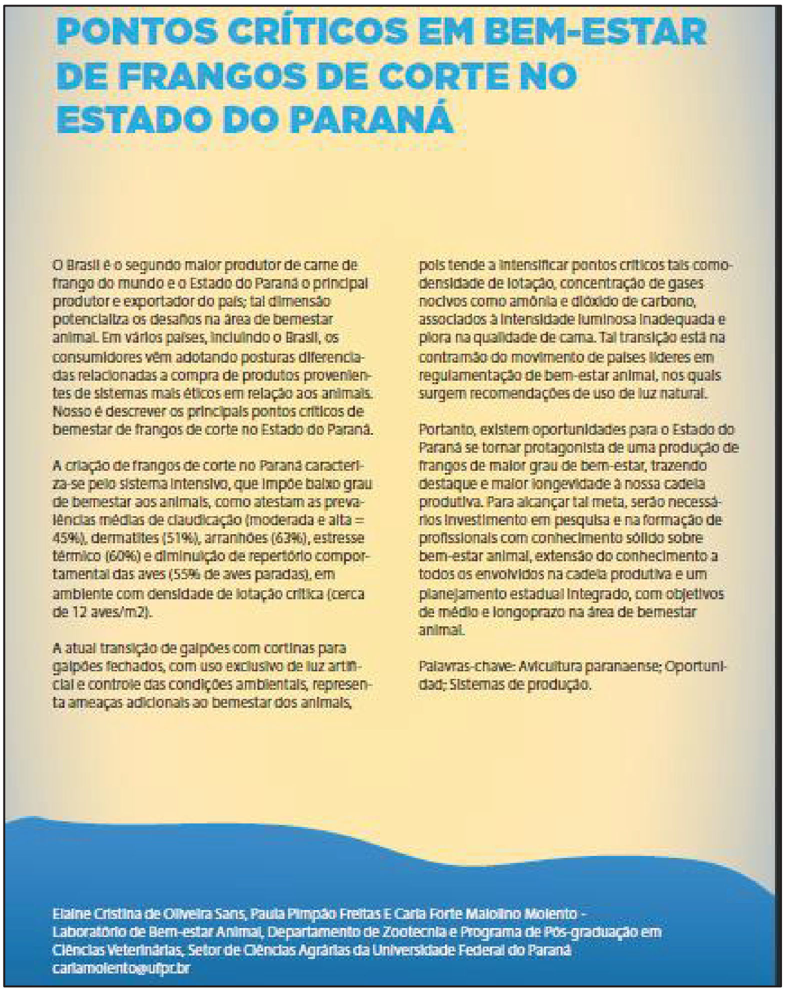
APPENDIX I – ABSTRACT: PONTOS CRÍTICOS EM BEM-ESTAR DE FRANGOS DE CORTE NO PARANÁ (HORIZONTE PARANAENSE EM BEM-ESTAR ANIMAL)



PONTOS CRÍTICOS EM BEM-ESTAR DE FRANGOS DE CORTE NO ESTADO DO PARANÁ

O Brasil é o segundo maior produtor de carne de frango do mundo e o Estado do Paraná o principal produtor e exportador do país; tal dimensão potencializa os desafios na área de bemestar animal. Em vários países, incluindo o Brasil, os consumidores vêm adotando posturas diferenciadas relacionadas a compra de produtos provenientes de sistemas mais éticos em relação aos animais. Nosso é descrever os principais pontos críticos de bemestar de frangos de corte no Estado do Paraná.

A criação de frangos de corte no Paraná caracteriza-se pelo sistema intensivo, que impõe baixo grau de bemestar aos animais, como atestam as prevalências médias de claudicação (moderada e alta = 45%), dermatites (51%), arranhões (63%), estresse térmico (60%) e diminuição de repertório comportamental das aves (55% de aves paradas), em ambiente com densidade de lotação crítica (cerca de 12 aves/m2).

A atual transição de galpões com cortinas para galpões fechados, com uso exclusivo de luz artificial e controle das condições ambientais, representa ameaças adicionais ao bemestar dos animais, pois tende a intensificar pontos críticos tais como densidade de lotação, concentração de gases nocivos como amônia e dióxido de carbono, associados à intensidade luminosa inadequada e piora na qualidade de cama. Tal transição está na contramão do movimento de países líderes em regulamentação de bem-estar animal, nos quais surgem recomendações de uso de luz natural.

Portanto, existem oportunidades para o Estado do Paraná se tornar protagonista de uma produção de frangos de maior grau de bem-estar, trazendo destaque e maior longevidade à nossa cadeia produtiva. Para alcançar tal meta, serão necessários investimento em pesquisa e na formação de profissionais com conhecimento sólido sobre bem-estar animal, extensão do conhecimento a todos os envolvidos na cadeia produtiva e um planejamento estadual integrado, com objetivos de médio e longoprazo na área de bemestar animal.

Palavras-chave: Avicultura paranaense; Oportunidad; Sistemas de produção.

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APPENDIX II – ABSTRACT: WELFARE OF BROILER CHICKENS REARED UNDER TWO DIFFERENT HOUSE TYPES (ISAE 2020 – CONGRESO LATINOAMERICANO)

el costo total. Para el SGC, estos fueron de 76,29%, 20,94% y 2,77%. SGC mostró mayores costos con depreciaciones (+0,70%), mantenimiento (+0,09%) e identificación matricial (+0,34%). La ganancia anual por kg de lechón producido fue de R$ 1,48 a SGI y de R$ 1,44 en SGC. **Discusión:** SGC fue más costoso debido a que emplea más área para los corrales, identificación electrónica y alimentador automático, para mantener el rendimiento reproductivo, aumentando el costo de mantenimiento y depreciación. Es esencial para la viabilidad del sistema obtener indicadores zootécnicos similares entre los sistemas, para lo cual no es suficiente utilizar gestaciones colectivas: es necesario adaptar tecnologías de gestión. **Conclusión:** a pesar de que en este estudio el lechón producido en SGC aumentó los costos en 1,41% cuando comparado con el sistema convencional, aún presentó un margen de beneficio de 28,31%, siendo económicamente viable.

Palabras clave: Bienestar animal, costo de producción, porcicultura.
Keywords: Animal welfare, cost of production, pig farming.

Heterophil-lymphocyte ratio as animal welfare indicator using slaughterhouse samples

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Introduction: Heterophil-Lymphocyte Ratio (H:LR) is widely used as a chronic stress indicator in birds; however, it demands physical restraint of the animal for blood collection and, thus, is invasive. There is a shortage of information regarding the welfare diagnostic value of measuring H:LR in samples collected in slaughterhouses, with the advantage of disassociation the post-stunning blood collection from the invasiveness of the *in vivo* procedure. **Objective:** To compare H:LR between blood samples collected *in vivo* and during slaughter, to determine the viability of H:LR as a chronic welfare indicator for laying hens from samples collected during slaughter. **Methodology:** Two blood collections from 25 Hisex Brown and 25 Dekalb Brown birds were planned, one on-farm sample taken *in vivo* from the ulnar vein, and a second slaughterhouse sample collected during the bleeding, after stunning and jugular vein sectioning. Eleven birds were not located in the slaughterhouse, hence results from 39 pairs of samples are presented. Blood Wright's stained film slides were counted for leukocytes and their H:LR calculated. **Results:** On-farm and slaughterhouse samples presented, respectively, H:LR of 0.73±0.3 and 1.29±0.6 (P<0.001). The linear correlation of *in vivo* and post-stunning H:LR was weak (r=0.13; P=0.42). **Discussion:** Results suggest the existence of important stressors between the sampling moments, and a relevant H:LR diagnostic power for the negative impact of this period to bird welfare. The difference in H:LR values may be related to cell redistribution the blood vessel lumen and perhaps also the time between the pre-slaughter stressor exposition and stunning allowed for the development of an increased H:LR, as the 16 hours of pre-slaughter and slaughter procedures may have been sufficient to cause such a change. **Conclusion:** H:LR from slaughterhouse blood samples are not useful to evaluate chronic stress during laying hen on-farm lives. However, the comparison of the H:LR between the two groups of samples suggests diagnostic power for the acute negative impacts of pre-slaughter and slaughter procedures on hen welfare, justifying further research.

Keywords: Birds, leukocytes, slaughterhouse, stress

Welfare of broiler chickens reared under two different house types

Bienestar de pollos de engorde criados en dos tipos de galpones

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Introduction: The poultry industry involves billions of animals and, thus, constitutes a priority for animal welfare. **Objective:** To compare closed-sided (CS) and open-sided (OS) industrial houses regarding broiler chicken welfare in Southern Brazil. **Methodology:** Ten flocks in each house type were evaluated. Relevant measures were organised into four groups: 1) health indicators: contact dermatitis on the breast and abdominal areas (CDE), bird soiling (BSO), foot pad dermatitis (FPD), hock burn (HBU), lameness (LAM), fractures (FRA), bruising (BRU), scratches (SCR), dead on arrival (DOA), diseases (DIS); 2) environmental indicators: relative humidity (RHU), temperature (TEM), air velocity (AVE), ammonia (NH_3), carbon dioxide (CO_2), light intensity (LIN), litter moisture (LMO); 3)

APPENDIX III – PAPER: WELFARE OF BROILER CHICKENS REARED UNDER TWO DIFFERENT TYPES OF HOUSING (ANIMAL WELFARE JOURNAL)

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Welfare of broiler chickens reared under two different types of housing

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Abstract

We compared closed- and open-sided industrial houses with respect to the welfare of broiler chickens in southern Brazil. Ten flocks from each design were evaluated and measures divided into the following categories: i) bird health: contact dermatitis on the breast and abdominal areas, bird soiling, foot-pad dermatitis, hock burn, lameness, fractures, bruising, scratches, dead on arrival, diseases; ii) environmental measurements: relative humidity, temperature, air velocity, ammonia (NH_3), carbon dioxide (CO_2), light intensity, litter moisture; iii) behaviour: bird behaviour, touch test; and iv) affective states: qualitative behaviour assessment. Closed-sided houses showed worse contact dermatitis on the breast and abdominal areas, lower exploratory behaviour prevalence, higher NH_3 (11.2 [± 6.8] vs 7.5 [± 3.9] ppm) and CO_2 (1,124.9 [± 561.5] vs 841.0 [± 158.0] ppm), lower light intensity (6.9 [± 6.3] vs 274.2 [± 241.9] lux), while open-sided houses had a higher prevalence for scratches and panting behaviour, and lower air velocity (2.1 [± 0.7] vs 1.1 [± 1.0] m s^{-1}). Stocking densities of 13.9 (± 0.4) and 12.0 (± 0.3) per m^2 for closed- and open-sided houses, respectively, likely influenced some results. All values shown are means (± SD). Even though open-sided houses presented fewer animal welfare restrictions (according to five indicators as opposed to three for closed-sided houses), both revealed important welfare problems, evidenced by poor environmental indicators, behavioural restrictions and injuries.

Keywords: *animal welfare, behaviour, dark-house, semi-climatised, slaughter, summer/autumn*

Introduction

Poultry is the most traded livestock species in the world, in terms of numbers of animals involved and meat tonnage, and Brazil is one of the leading producers and exporters. In 2020, around 5.9 billion birds were slaughtered (IBGE 2021) in Brazil, and the country produced 13.8 million tons of poultry meat, behind only the US (with 20.2 million tons) and China (14.6 million tons) (ABPA 2021). Due to the numbers of animals involved, poultry production becomes a major priority regarding animal welfare initiatives (Broom 2001; Rowe *et al* 2019). Improvements may stem from consumer and market pressure, company interests, new policies, funding availability, country and regional specificities and climate as well as individual specifications on-farm, such as house design and management.

No standard system is in place for raising broiler chickens in developing countries. However, there are concerns as regards striking a balance amongst farm maintenance conditions, animal welfare and production sustainability (Lima *et al* 2020). The Brazilian poultry industry utilises multiple systems with different house sizes and partial or absolute control over indoor environmental conditions. Most Brazilian broiler chickens are reared in open-sided poultry houses, so-called conventional and semi-climatised houses, with fans and access to natural lighting, combined with adjustable polypropylene curtains (Paranhos da Costa *et al* 2017). Closed-sided houses are fully enclosed by fixed curtains or walls and thermal insulation panels (Olanrewaju *et al* 2010) and usually equipped with negative pressure and evaporative cooling systems, exhaust fans and sprinklers, and exclusive artificial lighting (Olanrewaju *et al* 2010; Abreu & Abreu 2011; Baracho *et al* 2018). There are concerns about the lighting: for example, 75% of animal welfare experts studied by Rioja-Lang *et al* (2020) agreed on the potential negative impact of artificial lighting regimes on poultry welfare; there is also concern from consumers regarding lighting (Vanhonacker *et al* 2009). A number of authors recognise the importance of light, especially natural lighting, and offer recommendations for the inclusion of windows in closed-sided poultry house designs

APPENDIX IV – PAPER: WELFARE OF BROILER CHICKENS REARED IN TWO DIFFERENT INDUSTRIAL HOUSE TYPES DURING THE WINTER SEASON IN SOUTHERN BRAZIL (BRITISH POULTRY SCIENCE)

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Welfare of broiler chickens reared in two different industrial house types during the winter season in Southern Brazil

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ABSTRACT

1. The following trial compared broiler chicken welfare in closed-sided (CS) *versus* open-sided (OS) industrial house types during the winter season in the South of Brazil.
2. Ten flocks in each house type were evaluated as follows: a) bird health: contact dermatitis on the breast and abdominal areas (CDE), bird soiling (BSO), footpad dermatitis (FPD), hock burn (HBU), lameness (LAM), fractures (FRA), bruising (BRU), scratches (SCR), dead on arrival (DOA), and diseases (DIS); b) house environmental measurements: relative humidity (RHU), temperature (TEM), air velocity (AVE), illuminance (ILL), ammonia concentration (NH_3), and carbon dioxide concentration (CO_2), and c) bird behaviour and affective states: bird behaviour (BBE), touch test (TTE), and qualitative behaviour assessment (QBA).
3. Statistical analyses were based on regression models for CDE, BSO, FPD, HBU, LAM and generalised linear models for DOA, FRA, BRU, SCR, and DIS. The Mann–Whitney test was used for RHU, TEM, AVE, ILL, NH_3, CO_2, and the t-test for TTE and LMO, with a specific regression model for BBE data and Principal Component Analysis for QBA.
4. According to odds ratio for worse scores for CS relative to OS, birds were less likely to have severe scores for CDE ($P = 0.040$ and $P = 0.007$), BSO ($P = 0.031$, $P = 0.016$, and $P = 0.038$), and HBU ($P = 0.017$), and had higher median values for AVE (2.3, 0.0–7.8 m s^{-1} *vs.* 0.0, 0.0–4.3 m s^{-1}), lower NH_3 concentration (9.0, 0.0–64.0 ppm *vs.* 12.0, 0.0–60.0 ppm) and TTE scores (98, 96–100 *vs.* 67, 25–100). Worse results were observed in CS houses for higher stocking density (13.8 ± 0.2 birds/m^2 *vs.* 12.0 ± 0.2 birds/m^2), RHU (74.5, 50.7–99.9% *vs* 72.3, 47.4–99.9%), and TEM (23.9, 14.6–29.2°C *vs.* 21.7, 12.9–30.1°C), lower ILL (16.0, 1.0–60.0 lx *vs.* 161.0, 8.0–2380.0 lx), less drinking ($P = 0.007$), more inactive behaviour ($P < 0.001$) and lower positive emotions, according to QBA ($P = 0.028$).
5. In the studied region and season, CS houses seemed to offer fewer welfare problems in terms of the health indicators; however, OS houses showed fewer behavioural restrictions and higher positive emotional states.

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KEYWORDS
Aviary; behaviour; emotional state; environment; health; poultry; slaughter

Introduction

Animal welfare is a current and increasingly relevant issue in animal production. The importance of this is growing due to greater awareness of both markets and consumers around the world which demand more ethical production systems which are less harmful to animals (EU 2015; WAP 2016; Queiroz et al. 2018; Alonso et al. 2020).

The poultry meat industry involves a large number of chickens worldwide; therefore, there is a high priority for improving animal welfare in this context (Rowe et al. 2019). In 2019, Brazil was the third largest producer of poultry meat with 13.2 million tons, after the United States of America with 19.9 million tons and China with 13.7 million tons. Brazil was the number one exporter (4.2 million tons; ABPA 2020). This same year, the Brazilian industry sent around 5.8 billion broiler chickens to slaughter, with the State of Paraná being the highest in numbers of slaughtered animals and Santa Catarina, where this study was conducted, as the second highest, the latter being equivalent to 15.4% of national total (ABPA 2020; IBGE 2020).

Brazilian poultry production is distributed across all regions of Brazil, and different types of barn are employed as farm units, which may be categorised as conventional, semi- and fully acclimatised houses. The conventional and semi-climatised barns are generally characterised by open sides with curtains and artificial or natural light (or both). Of these two house types, only the semi-climatised barns are equipped with positive pressure fans and sprinklers. The Brazilian climatised barn is characterised by artificial lighting, negative pressure and evaporative cooling systems, exhaust fans and sprinklers, and fully curtain-closed sides. Curtains may be double and have different colours, such as black, blue, green or yellow (Baracho et al. 2018; Lima and Silva 2019). Although the reason for using closed-sided barns is to provide better control of internal environmental conditions, open-sided barns may present better air quality due natural ventilation (Lima et al. 2020; Sans et al. in press). Likewise, the absence of natural light in closed-sided barns can limit behavioural expression in birds (Bailie et al. 2018; Lima and Silva 2019). On the other hand, conventional and semi-climatised poultry houses may increase bird suffering due to thermal stress (Lima and Silva 2019). Thus, it is important to study which is house design fosters a positive association between animal welfare and environmental conditions (Mazzuco et al. 2019), through the monitoring of animal welfare indicators.

The assessment of animal welfare involves diverse elements which, when considered together, contribute to an understanding of issues in each production system. Some

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APPENDIX V – PAPER: IN-BARN HETEROGENEITY OF BROILER CHICKEN WELFARE IN TWO INDUSTRIAL HOUSE DESIGNS AND TWO SEASONS IN SOUTHERN BRAZILIAN SUBTROPICAL CLIMATE (LIVESTOCK SCIENCE)

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In-barn heterogeneity of broiler chicken welfare in two industrial house designs and two seasons in Southern Brazilian subtropical climate

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H I G H L I G H T S

• Geostatistics may to contribute to the adoption of strategies for animal welfare improvement.

A R T I C L E I N F O

Keywords:
Animal-based
Bird
Environment
Geostatistics
Health
Kriging maps

A B S T R A C T

The science of animal welfare is key to improving the life quality of billions of chickens, by supporting decisions through the assessment of environmental and animal-based indicators in different conditions. Our goal was to assess the variation of bird welfare within the same barn and whether this variation depends on barn type or season. We described and compared the in-barn heterogeneity of broiler chicken welfare in four closed-sided (CS) and 13 open-sided (OS) industrial poultry houses, during two different seasons (summer/autumn and winter). The measures were divided into two categories: 1) environmental indicators: relative humidity, temperature, air velocity, ammonia (NH_3) and carbon dioxide (CO_2) concentrations, and illuminance; 2) animal-based indicators: contact dermatitis on the breast and abdominal areas, bird soiling, footpad dermatitis, hock burn, and lameness. The results of assessments in 30 equidistant locations, covering the whole inside area of each barn, were organized into kriging maps. Linear regression and generalized models were fitted, considering predictor variables and the interaction effect between them; the Tukey test was used for the multiple comparisons of means. We used geostatistical modelling for continuous and discrete data for environmental and animal-based measurements, respectively. In-barn heterogeneity was observed for the prevalence of environmental and animal-based problems. There was a pattern for the spatial distribution, heading from the house centre to the West end of both house types, with worse results for three environmental indicators (higher temperature, and NH_3 and CO_2 concentrations) and three animal-based indicators (higher prevalence of hock burn, bird soiling and footpad dermatitis). In CS, illuminance was very restrictive (4.4 to 6.7 lx) when compared to OS houses (119.8 to 145.3 lx); in both house types the prevalence of lameness was high (50.9 to 78.0%), even though both illuminance and lameness prevalence were evenly distributed inside all houses. The kriging maps allowed for the identification of worse welfare problems in the West direction, which in CS means near exhaust fans and in OS houses the direction of positive-pressure mechanical ventilation by fans. Our results show that attention is needed for the variation of bird welfare conditions inside each barn, and allow for the adoption of strategies to spread best conditions throughout the internal barn area in both house designs. Principally, the original findings on in-barn bird welfare heterogeneity suggest the relevance of constant bird welfare monitoring in key locations within the barns, minimally for the indicators with known different in-barn spatial distributions.

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APPENDIX VI – ABSTRACT: IN-BARN HETEROGENEITY OF BROILER CHICKEN WELFARE IN TWO INDUSTRIAL HOUSE DESIGNS AND TWO SEASONS IN SOUTHERN BRAZILIAN SUBTROPICAL CLIMATE (UFAW 2021)

P93 Recent advances in animal welfare science VIII
Virtual UFAW Animal Welfare Conference, 29th- 30th June 2021

IN-BARN HETEROGENEITY OF BROILER CHICKEN WELFARE IN TWO INDUSTRIAL HOUSE DESIGNS AND TWO SEASONS IN SOUTHERN BRAZILIAN SUBTROPICAL CLIMATE

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The science of animal welfare is key to improving the life quality of billions of chickens, by supporting decisions through the assessment of environmental and animal-based indicators in different conditions. We compared four closed-sided (CS) and 13 open-sided (OS) industrial poultry houses regarding broiler chicken welfare, during summer/autumn and winter seasons, concerning bird location inside the house. The measures were divided into two categories: 1) environmental indicators: relative humidity, temperature, air velocity, ammonia (NH_3), and carbon dioxide (CO_2) concentrations, and illuminance; 2) health indicators: contact dermatitis on the breast and abdominal areas, bird soiling, footpad dermatitis, hock burn, and lameness. Assessments in 30 equidistant locations, covering the whole inside area of each barn, were organized into kriging maps. Linear regression and generalized models were adjusted, considering predictor variables and the interaction effect between variables; the Tukey test was used for the multiple comparisons of means. We used geostatistical modelling for continuous and discrete data for environmental measurements and bird health, respectively. In-barn spatial distribution heterogeneity was observed for the prevalence of both environmental and health indicators. In addition, there was a pattern for the spatial distribution heading from the house centre to the West end of both house types, with worse results for three environmental indicators (temperature, NH_3 and CO_2 concentrations) and three health indicators (hock burn, bird soiling, and footpad dermatitis). Illuminance was very restrictive in CS (4.4 to 6.7 lx) when compared to OS houses (119.8 to 145.3 lx) and the prevalence of lameness was high (50.9 to 78.0%), even though both indicators were evenly distributed inside the two house types studied. The kriging maps allowed for the identification of worse welfare problems in the West direction, which in CS houses means near exhaust fans and in OS houses the direction of positive-pressure mechanical ventilation by fans. These results may contribute to the adoption of strategies for animal welfare improvement, taking corrective actions in both house designs, with the goal of spreading best welfare conditions throughout the internal barn area.

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RECENT ADVANCES IN ANIMAL WELFARE SCIENCE VIII
Virtual UFAW Animal Welfare Conference
29th – 30th June 2021

IN - BARN HETEROGENEITY OF BROILER CHICKEN WELFARE IN TWO INDUSTRIAL HOUSE DESIGNS AND TWO SEASONS IN SOUTHERN BRAZILIAN SUBTROPICAL CLIMATE

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INTRODUCTION

The science of animal welfare is key to improving the life quality of billions of chickens, by supporting decisions through the assessment of environmental and animal-based indicators in different conditions. The aim of this study was to compare four closed-sided (CS) and 13 open-sided (OS) industrial poultry houses regarding broiler chicken welfare, during summer/autumn and winter seasons, concerning bird location inside the house.

MATERIAL & METHODS

The measures were divided into two categories: 1) environmental indicators such as relative humidity, temperature, air velocity, ammonia (NH_3), carbon dioxide (CO_2) concentrations, illuminance; 2) animal indicators such as contact dermatitis on the breast and abdominal areas, bird soiling, footpad dermatitis, hock burn, and lameness. Assessments in 30 equidistant locations, in both house types (Figure 1), were organized into kriging maps.

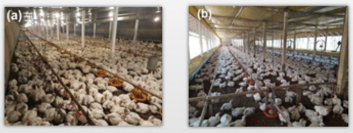

Figure 1. Closed-sided (a) and open-sided (b) houses.

RESULTS

In-barn spatial distribution heterogeneity was observed for the prevalence of both environmental and animal indicators. There was a pattern for the spatial distribution heading from the house centre to the West end of both house types, with worse results for three environmental indicators, such as temperature, NH_3 and CO_2 concentrations, and three animal-based indicators, such as hock burn, bird soiling, and footpad dermatitis (Figure 2; QR code).

Illuminance was restrictive in CS (4.4 to 6.7 lx) than OS houses (119.8 to 145.3 lx), and prevalence of lameness was high (50.9 to 78.0%), even though both indicators were evenly distributed inside both houses.

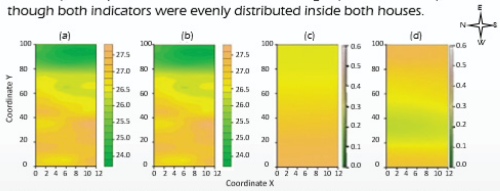

Figure 2. Spatial distribution of temperature (T °C) in CS houses during summer/autumn (a) and winter (b); and absence or presence of footpad dermatitis in CS (c) and OS houses (d), during summer/autumn.

DISCUSSION

The results suggest that there was absence of good conditions in some animal-based and environmental indicators, within both house types, that may directly influence on bird welfare. Results of environmental indicators, such as temperature, NH_3 and CO_2 concentrations were coherent with the same barn area, with greatest prevalences for hock burn, bird soiling and footpad dermatitis.

CONCLUSIONS

The kriging maps allowed for the identification of worse welfare problems in the West direction, which in CS houses means near exhaust fans and in OS houses the direction of positive-pressure mechanical ventilation by fans. These results may contribute to the adoption of strategies for animal welfare improvement, taking corrective actions in both house designs, and spreading best welfare conditions throughout the internal barn area.

Acknowledgements
CAPES

ANNEX I – ANIMAL USE ETHICS COMMITTEE (104/2017)

UFPR 100 Anos 1912 2012

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

CERTIFICADO

Certificamos que o protocolo número 104/2017, referente ao projeto **"Motivação em frangos de corte e estratégias de melhoria em seu bem-estar"**, sob a responsabilidade de **Carla Forte Maiolino Molento** – 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), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DO SETOR DE CIÊNCIAS AGRÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ - BRASIL, com grau 2 de invasividade, em reunião de 04/12/2017.

Vigência do projeto	Janeiro/2018 até Março/2019
Espécie/Linhagem	*Gallus gallus domesticus* (ave)
Número de animais	120.350
Peso/Idade	0.03 a 3.5 kg / 1 a 45 dias
Sexo	Ambos
Origem	Aviários comerciais da BRF Brasil Foods em Toledo, Paraná

CERTIFICATE

We certify that the protocol number 104/2017, regarding the project **"Motivation in broiler chicken and strategies to improve their welfare"** under **Carla Forte Maiolino Molento** 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, of 8 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 the ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES CAMPUS OF THE UNIVERSIDADE FEDERAL DO PARANÁ (Federal University of the State of Paraná, Brazil), with degree 2 of invasiveness, in session of 12/04/2017.

Duration of the project	January/2018 until March/2019
Specie/Line	*Gallus gallus domesticus* (bird)
Number of animals	120.350
Wheight/Age	0.03 to 3.5 kg / 1 to 45 days
Sex	Both
Origin	Commercial aviaries from BRF Brasil Foods in Toledo, Paraná

Curitiba, 4 de dezembro de 2017.

Chayane da Rocha

Chayane da Rocha

Coordenadora CEUA-SCA

ANNEX II - ANIMAL USE ETHICS COMMITTEE (046/2018)

UFPR 100 Anos 1912 - 2012

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

CERTIFICADO

Certificamos que o protocolo número 046/2018, referente ao projeto **"Do ponto de vista dos frangos de corte: bem-estar em dois diferentes tipos de galpões"**, sob a responsabilidade de **Carla Forte Maiolino Molento** – 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), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DO SETOR DE CIÊNCIAS AGRÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ - BRASIL, com grau 2 de invasividade, em reunião de 05/07/2018.

Vigência do projeto	Julho/2018 até Dezembro/2020
Espécie/Linhagem	*Gallus gallus domesticus* (ave)/Comercial
Número de animais	2.400.000
Peso/Idade	0,03 – 3,5 kg/1 - 45 dias
Sexo	Macho e fêmea
Origem	Granja comercial BRF S.A., Brasil

CERTIFICATE

We certify that the protocol number 046/2018, regarding the project **"From a broiler chicken's point of view: welfare in two different housing systems"** under **Carla Forte Maiolino Molento** 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, of 8 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 the ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES CAMPUS OF THE UNIVERSIDADE FEDERAL DO PARANÁ (Federal University of the State of Paraná, Brazil), with degree 2 of invasiveness, in session of 05/07/2018.

Duration of the project	July/2018 until December/2020
Specie/Line	*Gallus gallus domesticus* (bird)/Comercial
Number of animals	2.400.000
Wheight/Age	0.03 – 3.5 kg/1 - 45 days
Sex	Male and female
Origin	BRF S.A. commercial farm, Brazil

Curitiba, 05 de julho de 2018.

Chayane da Rocha

Chayane da Rocha

Coordenadora CEUA-SCA

ANNEX III – DECLARAÇÃO DE BOLSISTA CAPES

MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIÊNCIAS AGRÁRIAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO CIÊNCIAS
VETERINÁRIAS - 40001016023P3

DECLARAÇÃO DE BOLSISTA

Data da emissão: 27/04/2021

Declaramos que ELAINE CRISTINA DE OLIVEIRA SANS (CPF), é aluna bolsista pela Agência CAPES - DS no curso de Doutorado do Programa de Pós-Graduação em CIÊNCIAS VETERINÁRIAS da UFPR, sob o número sendo beneficiado mensalmente desde 01/04/2017, com previsão de fim da bolsa em 30/06/2021

CARLA FORTE MAIOLINO MOLENTO
Coordenação do Programa de Pós Graduação em
CIÊNCIAS VETERINÁRIAS

RUA DOS FUNCIONÁRIOS, 1540 - CURITIBA - Paraná - Brasil
CEP 80035050 - Tel: (41) 3350-5621 - E-mail: cpgcv@ufpr.br
https://www.prppg.ufpr.br/siga/visitante/autenticacao.jsp - Código para autenticação: ulMlbp1m

ANNEX IV – ACORDO DE PARCERIA ENTRE LABEA, WORLD ANIMAL PROTECTION E REDE BRASILEIRA PARA RECEBIMENTO DE APOIO FINANCEIRO PARA COLETA DE DADOS

ACORDO DE PARCERIA

Pelo presente instrumento particular que celebram, de um lado **SOCIEDADE MUNDIAL DE PROTEÇÃO ANIMAL – WSPA BRASIL**, sociedade sem fins lucrativos, devidamente inscrita no CNPJ/MF sob nº [redacted] com sede na Av. Paulista, 453 – conj. 32 e 34, São Paulo/SP, neste ato representada por seu representante legal abaixo identificado, doravante denominada simplesmente **WSPA** e, de outro lado,

REDE BRASILEIRA PARA O DESENVOLVIMENTO DA METROLOGIA, TECNOLOGIA E QUALIDADE, pessoa jurídica, de direito privado sem fins lucrativos, devidamente inscrita no CNPJ/MF sob nº- [redacted] 33, com sede na cidade e comarca de Curitiba, Estado do Paraná, na Avenida Comendador Franco, 1341, Cep: 80.215-090, neste ato representada na forma de seu Estatuto Social, doravante designada simplesmente **PARCEIRA;**

CARLA FORTE MAIOLINO MOLENTO, brasileira, médica veterinária, portadora da cédula de identidade RG [redacted], inscrita no CPF nº [redacted] residente domiciliada na Cidade de Curitiba, Estado do Paraná, na qualidade de **ANUENTE**.

Considerando que a WSPA pretende que a PARCEIRA conduza os estudos por meio da ANUENTE, os partícipes resolvem, de pleno direito, firmar o presente Acordo de Parceria, regendo-se pelas seguintes cláusulas e condições:

CLÁUSULA PRIMEIRA - DO OBJETO

1.1. Constitui objeto deste Acordo o estabelecimento dos termos e condições da parceria entabulada entre as Partes, que tem por objeto o desenvolvimento de um estudo comportamental e de bem-estar em frangos de corte com o objetivo de demonstrar sobre a perspectiva do frango o bem-estar em dois diferentes tipos de galpão.