UNIVERSIDADE FEDERAL DO PARANÁ

PRISCILLA REGINA TAMIOSO

# INDICATORS OF POSITIVE EMOTIONAL STATES IN SHEEP AND PERCEPTION OF SHEEP WELFARE AND SENTIENCE BY BRAZILIAN AND FRENCH CITIZENS

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# INDICATORS OF POSITIVE EMOTIONAL STATES IN SHEEP AND PERCEPTION OF SHEEP WELFARE AND SENTIENCE BY BRAZILIAN AND FRENCH CITIZENS

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Supervisor: Professor Dr. Carla Forte Maiolino Molento

Guidance Committee: Professors Dr. Ricardo Guilherme D'Otaviano de Castro Vilani and Dr. Marcelo Beltrão Molento

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CURITIBA, 08 de Dezembro de 2017.

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Presidente da Banca Examinadora

FRANK ANDRE MAURICE TUYTTENS
Avaliador Externo

SIMONE TOSTES DE OLIVEIRA STEDILE
Avaliador Interno

VANESSA CARLI BONES
Avaliador Externo

CRISTINA SANTOS SOTOMAIOR
Avaliador Externo

To the animals

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There is no fundamental difference between man and animals in their ability to feel pleasure and pain, happiness, and misery.

**Charles Darwin**

## RESUMO

O objetivo desta tese foi avaliar indicadores comportamentais e fisiológicos de ovinos submetidos a manejo positivo. Objetivou-se também estudar a percepção de cidadãos brasileiros e franceses em relação a bem-estar e senciência animal, com enfoque em ovinos. A tese está organizada em sete capítulos: (1) Apresentação; (2) Comportamento e temperatura da superfície corporal como indicadores de bem-estar em ovinos selecionados, regularmente escovados por um observador familiar; (3) Indução de emoções positivas: respostas comportamentais e cardíacas à presença humana e à escovação em ovelhas selecionadas para alta e baixa reatividade social; (4) A reatividade emocional altera as respostas relaxantes de ovelhas escovadas?; (5) Atitudes de ovinocultores do sul do Brasil acerca de bem-estar e senciência em animais; (6) Percepção de bem-estar e senciência em ovinos por cidadãos brasileiros e franceses; (7) Considerações finais. O capítulo sobre o estudo de indicadores comportamentais e de temperatura de ovinos submetidos à escovação mostrou que posturas de orelhas e presença de olhos semicerrados podem ser consideradas indicadores úteis na avaliação de emoções positivas em ovinos, bem como temperaturas de cernelha e nasal. O efeito racial também forneceu dados importantes sobre a interpretação de posturas de orelha. O capítulo sobre o estudo de indicadores comportamentais e cardíacos de ovelhas mais (R+) e menos (R-) reativas ao isolamento social indicou que a presença humana e a escovação induziram um estado relaxante nos animais, especialmente durante a escovação, e em ovelhas R+. Ao analisar o efeito de barreiras físicas separando o animal teste de membros do grupo sobre as respostas comportamentais e cardíacas de ovelhas escovadas R+ e R-, os resultados indicaram que a presença das mesmas pode ter provocado respostas negativas em ambas as linhagens genéticas, por meio de dados de posturas de orelhas e frequência cardíaca, e confirmaram que a escovação eliciou estado relaxante nas ovelhas. A partir dos resultados do capítulo sobre o estudo das atitudes de ovinocultores do sul do Brasil em relação a questões de bem-estar animal, foi possível inferir que o conhecimento dos produtores sobre bem-estar animal, atitudes acerca de senciência animal e reconhecimento do sofrimento em ovinos devido a práticas específicas podem ser melhorados. Os ovinocultores associaram bem-estar animal principalmente com nutrição e a experiência na indústria de ovinos teve um efeito negativo sobre as atitudes dos produtores em relação a bem-estar animal. O capítulo sobre o estudo da percepção dos cidadãos brasileiros e franceses sobre bem-estar e senciência animal mostrou diferentes percepções por cidadãos comuns de Curitiba, Paraná, Brasil e Clermont-Ferrand, Theix, França. Cidadãos de Curitiba apresentaram maior percepção sobre as questões de bem-estar avaliadas, sobretudo quanto ao sofrimento em ovinos durante práticas de manejo. Quando cidadãos comuns, veterinários, biólogos e zootecnistas de Curitiba foram comparados, observou-se que cidadãos comuns e biólogos tiveram maiores percepções sobre os temas. Nossos resultados colaboraram para o estudo de indicadores comportamentais e fisiológicos de bem-estar positivo em ovinos e para o conhecimento de percepções de especialistas e não especialistas em bem-estar e senciência animal.

Palavras-chave: Bem-estar positivo. Comportamento. Ovinos. Percepção. Pesquisa de opinião. Reatividade emocional. Respostas autonômicas.

## ABSTRACT

The objective of this thesis was to assess behavioral and physiological indicators of sheep submitted to positive handling. Furthermore, we aimed to study the perception of Brazilian and French citizens regarding animal welfare and sentience, with special attention to sheep. The thesis is organized into seven chapters: (1) Presentation; (2) Behavior and body surface temperature as welfare indicators in selected sheep regularly brushed by a familiar observer; (3) Inducing positive emotions: behavioural and cardiac responses to human and brushing in ewes selected for high vs low social reactivity; (4) Does emotional reactivity alter the relaxing responses of brushed ewes?; (5) Attitudes of South Brazilian sheep farmers to animal welfare and sentience; (6) Perception of animal sentience by Brazilian and French citizens; (7) Final considerations. The chapter on the study of behavioral and temperature indicators of sheep submitted to brushing showed that ear postures and half-closed eyes may be considered useful indicators when assessing positive emotions in sheep, as well as withers and nasal temperatures. The breed effect has also provided important data on the interpretation of ear postures. The chapter on the study of behavioral and cardiac indicators of highly (R+) and lowly (R-) reactive sheep to social isolation indicated that both human presence and brushing induced a relaxing state in sheep, especially during brushing, and in R+ animals. When analyzing the effect of physical barriers separating the test animal from group members on behavioral and cardiac responses of R+ and R- brushed sheep, the findings indicated that such barriers might have elicited negative responses in both genetic lines, through ear posture and heart rate data, and confirmed that brushing elicited a relaxing state on sheep. From the results of the chapter on the assessment of attitudes of South Brazilian sheep farmers to animal welfare issues, it was possible to infer that their knowledge of animal welfare, attitudes to animal sentience and recognition of sheep suffering due to specific procedures might be improved. Sheep farmers associated animal welfare mainly with animal nutrition and experience in the sheep industry had a negative effect over farmers' attitudes to animal welfare. The chapter on the study of Brazilian and French citizens' perception of animal welfare and sentience showed different perceptions by ordinary citizens from Curitiba, Parana, Brazil and Clermont-Ferrand, Theix, France. Citizens from Curitiba showed higher perception of animal welfare issues, mainly on sheep suffering during management procedures. When ordinary citizens, veterinarians, biologists and animal scientists of Curitiba were compared, it was found that ordinary citizens and biologists had higher perceptions of the subjects. Our results collaborated to the study of behavioral and physiological indicators of positive welfare in sheep and to the knowledge of perceptions of experts and non-experts toward animal welfare and sentience.



Keywords: Positive welfare. Behavior. Sheep. Perception. Survey. Emotional reactivity. Autonomic responses.

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# 1. PRESENTATION

Over the last years, there has been a considerable expansion in the publication of scientific studies on sentience of several species, mainly mammals. A sentient being has some degree of consciousness and/or awareness and the ability to evaluate the actions of others in relation to itself, as well as remember these actions and consequences (BROOM, 2010). It is believed that the attribution of sentience to animals may influence the way society perceives them. Allied with scientific studies, the recognition of emotional abilities in animals may contribute to society's concern and interest in ethics and welfare issues (MAYFIELD et al., 2007). The present work aims, then, to study behavioral and physiological indicators of positive welfare in sheep, through chapters 2, 3 and 4, and the perception of respondents from Brazil and France concerning animal welfare and sentience, through chapters 5 and 6.

Chapter 2 presents data on behavioral and body surface temperature as positive welfare indicators in sheep submitted to brushing. Such chapter was published by Priscilla Regina Tamioso, Daniel Santiago Rucinque, a Master's student of Labea - Animal Welfare Laboratory, UFPR - Federal University of Parana, from 2014 to 2016, Cesar Augusto Taconeli, professor of the Department of Statistics of UFPR, Guilherme Parreira da Silva, a statistician of UFPR, and Carla Forte Maiolino Molento in Journal of Veterinary Behavior: Clinical Applications and Research (APPENDIX II). Besides this publication, Appendices III, IV, XIII, XIV, XV and XVI refer to related published texts.

Chapter 3 comprises the behavioral and cardiac indicators of highly (R+) and lowly (R-) reactive sheep to social isolation, submitted to brushing and human presence. This chapter was written by Priscilla Regina Tamioso, Carla Forte Maiolino Molento, Xavier Boivin, leader of the research team entitled Animal Behaviour and Welfare of the Herbivores Joint Research Unit at INRA - Institut National de la Recherche Agronomique, Clermont-Ferrand, Theix, France, Hervé Chandèze, Stéphane Andanson, Éric Delval, technicians from the Herbivores Joint Research Unit at INRA, Clermont-Ferrand/Theix, France, Dominique Hazard, geneticist of the Department of Genetics, INRA, Paris, France, Guilherme Parreira da Silva, Cesar Augusto Taconeli and Alain Boissy, senior researcher of the Herbivores Joint Research Unit at INRA, Clermont-Ferrand/Theix, France. Such paper was submitted

to publication in Applied Animal Behaviour Science Journal. Besides the manuscript, Appendices V, VI, XIII, XIV, XV and XVI refer to related published texts.

Chapter 4 refers to the study of the influence of emotional reactivity on behavioral and cardiac responses of R+ and R- Romane ewes submitted to brushing. This chapter was written by Priscilla Regina Tamioso, Alain Boissy, Xavier Boivin, Hervé Chandèze, Stéphane Andanson, Éric Delval, Dominique Hazard, Cesar Augusto Taconeli and Carla Forte Maiolino Molento, and it will be submitted to Animal Journal. Besides the manuscript, Appendices XIII, XIV, XV and XVI refer to related published texts. Results of chapters 3 and 4 were produced during experiments in Clermont-Ferrand-Theix, France and Roquefort, France, from June to October 2015, through the provision of a grant by the Brazilian Program Science Without Borders – CSF/CNPq (ANNEX I).

Chapter 5 presents data on the perception of South Brazilian sheep farmers toward animal welfare and sentience. The text was written by Priscilla Regina Tamioso, Paulo Ricardo Bittencourt Guimarães, professor of the Department of Statistics of UFPR and Carla Forte Maiolino Molento, and it was published in Ciência Rural Journal (APPENDIX VII). Besides this chapter, Appendices VIII and XVI refer to related published texts.

Chapter 6 describes and compares the perception of citizens from Curitiba, Parana, Brazil and Clermont-Ferrand, Theix, France in relation to sheep welfare and sentience. The chapter was written by Priscilla Regina Tamioso, Daniel Santiago Rucinque, Mara Miele, professor of the Cardiff School of Planning and Geography, Cardiff University, United Kingdom, Alain Boissy and Carla Forte Maiolino Molento, and it was submitted to Animal Welfare Journal. Besides this chapter, Appendices IX, X, XI, XII and XVI refer to related published texts.

Chapter 7 brings the main findings of the studies on behavioral and physiological indicators of sheep in response to gentle physical handling, and perception of Brazilian and French citizens regarding sheep welfare and sentience. Conclusions and contributions of the thesis are also presented in this chapter.

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MAYFIELD, L.E.; BENNETT, R.M.; TRANTER, R.B.; WOOLDRIDGE, M.J. Consumption of welfare - friendly food products in Great Britain, Italy and Sweden, and how it may be influenced by consumer attitudes to, and behaviour towards, animal welfare attributes. **International Journal of Sociology of Food and Agriculture**, v. 15, n. 3, p. 59-73, 2007.

# 2. BEHAVIOR AND BODY SURFACE TEMPERATURE AS WELFARE INDICATORS IN SELECTED SHEEP REGULARLY BRUSHED BY A FAMILIAR OBSERVER

## RESUMO

Pesquisas sobre emoções em animais usados para a produção de alimentos têm contribuído progressivamente para o bem-estar dos mesmos. Objetivou-se estudar as respostas comportamentais e de temperatura corporal de ovinos Dorper e White Dorper regularmente escovados nas regiões ventral do pescoço, laterais e cernelha. Os animais foram avaliados durante 3 minutos nas fases pré-escovação, escovação e pós-escovação. Vocalização, posturas e mudanças de posturas de orelha, olhos semicerrados e movimentação de cauda foram estudados. As temperaturas de cernelha (Tw), anal (Ta) e nasal (Tn) também foram registradas, por meio de um termômetro infravermelho. Vocalizações foram infrequentes ao longo das fases. Três posturas de orelha frequentemente expressadas pelos animais foram identificadas: levantada (R), horizontal (H) e para trás (B). Foi observada maior duração de R em vez de H antes do que durante a escovação. Quando as posturas B e H foram comparadas, notou-se que ovinos Dorper expressaram a postura B por mais tempo, principalmente durante do que após a escovação. Na comparação entre as posturas B e R, observou-se que ovinos expressaram a postura B por mais tempo durante do que antes e após a escovação; ovinos da raça Dorper e machos também expressaram a postura B por mais tempo. Os resultados sugerem maior expressão das posturas B e H durante o estímulo e que sua expressão pode estar associada a um estado positivo, relaxante nos animais. Nenhum resultado significativo foi encontrado para mudanças de postura de orelhas. Os animais apresentaram olhos semicerrados principalmente durante e após a escovação, em comparação com a fase pré-escovação, indicando que a escovação pode ter eliciado um estado relaxante nos animais, o que poderia ter persistido após o estímulo. Apenas 4 machos movimentaram suas caudas, principalmente quando escovados. Comportamentos de busca por atenção, incluindo seguir o observador, encostar-se na escova com a cabeça ou pescoço e esticar o pescoço durante a escovação também foram observados. Os resultados de temperatura corporal indicaram maior variação para a Tw em relação a Tn e Ta, que apresentaram variâncias semelhantes. Comparações indicaram que a Tw foi maior na fase pós do que na fase pré-escovação; a Tn também foi maior após a escovação, do que antes e durante, sugerindo aumento de Tw e Tn após o estímulo. Não foram encontradas diferenças significativas para a Ta. A escovação pode ter eliciado um estado positivo nos animais. Posturas de orelha e olhos semicerrados podem ser ferramentas úteis para avaliar emoções em ovinos, bem como as temperaturas de cernelha e nasal. Além disso, características raciais podem ser um efeito altamente significativo sobre a expressão de posturas de orelha em ovinos.

Palavras-chave: Bem-estar animal. Contato tátil. Emoção. Indicadores positivos. Ovinos. Temperatura nasal.

## ABSTRACT

Research on emotions in farm animals has increasingly contributed to their welfare. We aimed to study behavioral and body temperature responses of Dorper and White Dorper sheep regularly brushed on their ventral neck, lateral chest and withers. We performed 3-minute assessments in prebrushing, brushing, and postbrushing phases. Vocalization, ear postures and changes, half-closed eyes and tail wagging were assessed. We also recorded withers (Tw), anal (Ta) and nasal (Tn) temperatures with an infrared thermometer. Vocalization was infrequent throughout the phases. We identified 3 ear postures frequently performed by the animals: raised up (R), horizontal (H), and backward (B). We noted a longer duration of R rather than H posture before brushing than during brushing. By comparing B and H, we observed that Dorper sheep performed the B posture for longer, mainly during than postbrushing. When B and R were compared, sheep expressed the B posture for longer during brushing than both prebrushing and postbrushing phases; Dorpers and male sheep also performed the B posture for longer. The results suggest higher performance of B and H postures during the stimulus and that their expression might be associated with a positive, relaxing state of the animals. No significant result was found for ear changes. Sheep showed half-closed eyes mainly in brushing and postbrushing phases, in comparison with the prebrushing phase, indicating that brushing might have elicited a relaxing state in the animals, which might have persisted after the stimulus. Only 4 male sheep wagged their tails, mostly when brushed. We also noted attention-seeking behaviors, including following the observer, leaning against the brush with the head or neck, and stretching the neck when brushed. The results on body temperatures indicated higher variance for Tw in relation to Tn and Ta, which had similar variances. Pairwise comparisons indicated that Tw was higher in postbrushing than in the prebrushing phase; Tn was also higher in the postbrushing phase than in both prebrushing and brushing phases, suggesting an increase in Tw and Tn after the stimulus. No significant differences were reported for Ta. Brushing might have elicited a positive state in sheep. Ear postures and half-closed eyes may be useful tools for assessing emotions in sheep, as well as withers and nasal temperatures. Furthermore, breed may be a highly significant effect on the expression of ear postures in sheep.

Keywords: Animal welfare. Tactile contact. Emotion. Positive indicators. Sheep. Nasal temperature.

## 2.1 INTRODUCTION

Emotions in animals were described by Charles Darwin in his book “The expression of emotions in man and animals,” in 1872 in which the naturalist compared emotional expressions such as gestures, postures, and facial moves associated with specific contexts in human beings and several animal species. Darwin supported the idea that there is continuity between humans and animals regarding their emotional lives. There are many concepts involved in the study of emotions, the majority of which are founded on research involving humans (KLEINGINNA; KLEINGINNA, 1981). Emotions may be defined as short and intense affective responses to an event and include physiological, behavioral, cognitive, motivational, motor, and subjective components (DÉSIRÉ et al., 2006). According to Mendl, Burman and Paul (2010), the study of emotions in animals considers as important 2 dimensional approaches, valence and arousal. The former is related to positive and negative features of emotions and ranges from pleasant to unpleasant, whereas the latter denotes the activation of an emotion on a gradient ranging from low to high.

Although there is a growing body of research on animal emotions, much of the concern toward welfare is focused on negative affective states. However, a trend in publications centered on the assessment of positive experiences in animals has been observed lately. Boissy et al. (2007) stated that positive emotions should be used in animal welfare assessments. Situations of traditionally presumed positive valence include feeding and play; recent studies suggest that stroking, grooming, brushing, and other types of tactile contact may also elicit positive emotions in farm animals (SCHMIED; BOIVIN; WAIBLINGER, 2008; PROCTOR; CARDER, 2014). In ruminants, positive emotions are behaviorally expressed by high proportions of horizontal ear postures, fewer ear-posture changes, absent or infrequent vocalization and half-closed eyes, or low relative eye aperture (SCHMIED; BOIVIN; WAIBLINGER, 2008; REEFMANN et al., 2009a; REEFMANN; WECHSLER, GYGAX, 2009; PROCTOR; CARDER, 2014; COULON et al., 2015). There has also been an increasing interest in the study of temperature measured through infrared thermography and its association with animal emotional states. Infrared thermography is a noninvasive method to assess blood flow changes, which has been used to detect alterations in

emotional states in farm animals, with special attention to stressful events (STEWART et al., 2005). Negative situations are usually characterized by a drop in peripheral temperatures (STEWART et al., 2005). However, recent studies suggest that a reduction in nasal temperatures may be associated with perception of presumed positive situations, such as tactile interactions (PROCTOR; CARDER, 2015b).

Gentle tactile contact similar to intraspecific allogrooming may also elicit appeasing, relaxing responses (SCHMIED; BOIVIN; WAIBLINGER, 2008), in addition to reduced levels of fear of humans in livestock species (BOIVIN et al., 1998). In a recent research on brushing, Westerath, Gygax and Hillmann (2014) observed that, during the adaptation phase, some calves showed fear of the observers. However, during the experiments, all the animals showed a tendency in preference for the compartment with the brushing experimenter. They also exhibited alternative behavior in form of turning, more exploring, self-grooming, and vocalizing when not being brushed, suggesting that brushing was judged as positive by the animals.

Looking for best situations eliciting positive emotions in sheep, valuable information can be taken from their social behavior. Sheep maintain large gregarious groups and spend large amount of time in close proximity with conspecifics. In addition, tactile stimulation through licking plays an important role in lamb-ewe attachment during the first hours after birth (NOWAK et al., 1997). Lambs usually keep physical interactions with their mothers by sleeping in body contact, next to their mother's body or on top of her, or against the body of other lambs (NOWAK; BOIVIN, 2015); older members of a group may also be seen lying against the body of conspecifics. Such facts support the hypothesis that sheep may perceive gentle tactile stimuli as positive (REEFMANN; WECHSLER; GYGAX, 2009; COULON et al., 2015). Therefore, this work aimed to assess behavior and body surface temperatures as positive welfare indicators of sheep regularly brushed by a familiar observer.

## 2.2 MATERIAL AND METHODS

### 2.2.1 Animals and housing

The experiment was carried out between November 2014 and February 2015 (mean temperatures during the period: 25±4°C) at a commercial farm in São Luiz do Purunã, Paraná, South of Brazil, latitude -25.46° and longitude -49.72°, on a farm where sheep are raised for breeding purposes. Thirty-three sheep were assessed for the study: 25 Dorper (11 non-castrated males and 14 females at 19±6 months-old and weighing 69±7 kg) and 8 White Dorper (2 non-castrated males and 6 females at 21±4 months-old, with 64±5 kg). Dorper is a synthetic breed developed in South Africa from crosses between Dorset horn and blackhead Persian sheep breeds and White Dorper was developed from crosses between Dorper and Persian, and Dorset horn and Van Rooy sheep breeds (MILNE, 2000). Dorpers are white-bodied sheep with a blackhead and White Dorpers are completely white sheep. Body cover in both Dorper and White Dorper is short and smooth, consisting primarily of hair, with a slight mixture of wool on top of the body.

Animals were born on the farm where the research was conducted and all of them were reared under the same management conditions before, during, and after the experiments. Water was available *ad libitum*, and the feeder was filled twice a day (8 am and 5 pm) with hay and ration composed of cornmeal (200 g/animal/ day). The experiments were conducted at least 3 hours later (11 am) and before feeding (2 pm). The animals had outdoor access every day from 08:30 to 10:00 and from 16:00 to 17:00. The animals were used to different stock people and daily human contact (3 hours a day) because they were managed for breeding exhibitions. Brushing was not part of the regular management, and the assessed animals had contact with the procedure only twice a year. Dorper and White Dorper sheep were mixed within groups of 3 to 7 individuals, of the same sex within pens of 14 $m^2$ inside a building. The floor was covered in straw, and it was cleaned every day at 8 am and 5 pm. The procedures were approved by the Federal University of Paraná Ethics Committee on Animal Use - CEUA/SCA/UFPR, under protocol number 025/2014 (ANNEX II). After the experiments, the animals were kept on the farm of origin, according to the farm routine.

### 2.2.2 Selection of the animals

Sheep were tested for 3 consecutive weeks. The selection test occurred individually, 3 minute per animal. During the first week, a female observer (experimenter A, female, 165 cm, 56 kg) entered the pen and kept still, without trying any physical contact with the animals. During the second week, the experimenter A repeated the procedure and introduced a 20 cm × 11 cm slicker brush with fine wire bristles (1.2 cm length; see more details in the following), which was on her hand, but did not touch the animal. During the third week, the experimenter A tried to move the brush toward the sheep's body, to touch the body but not brush it. In the end, we selected the animals based on adaptation responses and their acceptance or approach toward the experimenter A and the brush. Four Dorper (2 females and 2 males) and 2 White Dorper sheep (2 females) exhibited avoidance behavior, that is, they moved away while being touched. These animals were removed from the experiment and the testing pens; they were kept with other sheep of the farm that did not belong to our study. In total, 27 sheep were studied.

### 2.2.3 Adaptation period

After selection, the animals were submitted to an adaptation period toward another experimenter, B, and the brushing procedure. Experimenter B (male, 155 cm, 60 kg) was responsible for assessing body surface temperatures responses and experimenter A, who participated in the selection test, brushed the animals. During all the sessions, experimenter B stayed out of the pen.

The adaptation period lasted 3 more consecutive weeks, 4 days of the week. Each animal was adapted toward the procedure and the brush for about 10 minutes per day. Pens were tested following the same order in every procedure, but individual sheep within a pen was assessed in a random order. The adaptation period ended once the animals became consistently relaxed with the procedure and the brush. A slicker brush was used which allowed the bristles to move freely through the sheep coat without catching on tangled fibers. Sheep were brushed in groups, in the following areas of the body: withers, lateral chest, and ventral neck. These body regions were selected because they were reported as preferred body regions to be groomed in cows (SCHMIED; BOIVIN; WAIBLINGER, 2008). Although allogrooming

is rather rarely seen in sheep (BOISSY et al., 2007), we presumed that such body regions would be preferred areas to be brushed in sheep, too. In fact, we observed that brushing these regions elicited behaviors such as following the observer, leaning against the brush with the head or neck, leaning against the observer's legs with the head and neck stretching, which may be related to positive affective states (WESTERATH; GYGAX; HILLMANN, 2014).

Brushing was performed 4 times at each body area, for 3 minutes on each animal; the order of regions to be brushed was standardized and consistent across days for all the animals. Brushing speed ranged between 30 to 50 brushing strokes/minute and touched the sheep skin. Brushing was initiated by the entrance of experimenter A in the home pen. Then, experimenter A sat on a bench, took notes on start time and sheep identification. If the sheep voluntarily approached the experimenter within 60 seconds, the experimenter A brushed the animal as previously described. If the animal did not approach the experimenter A, that is, the animal was still, experimenter A gently attempted to establish physical contact. During the experiments, if any other animal approached, experimenter A gently stepped away and continued brushing the focal sheep.

### 2.2.4 Procedure

Three days after finishing the adaptation period, brushing sessions were initiated. They were carried out each 15 days, for 3 consecutive days. Brushing sessions occurred as described for the adaptation period. Each animal was tested once.

### 2.2.5 Behavioral and physiological assessments

Behaviors were assessed in prebrushing, brushing, and postbrushing phases, using continuous recording. Each phase comprised 3 minute of assessment, totaling 9 minutes of observation. Prebrushing refers to the phase before brushing, the experimenter A was out of the pen; brushing was the phase in which the experimenter A submitted the animal to brushing the 3 body regions; and postbrushing refers to the phase of 3 minutes after the stimulus, the experimenter A was out of the pen.

A camera mounted on a tripod was placed above the home pen where the animal was brushed. The measured behavioral indicators were vocalization, ear postures and changes, presence of half-closed eyes (FIGURE 1) and tail wagging, assessed per phase. Vocalization was analyzed as number of times. Ear postures were categorized as described by Boissy et al. (2011): raised up (R), horizontal (H), backward (B), and asymmetric (A) ears. Total duration of each ear posture was assessed in seconds. The total number of ear-posture changes per phase was counted. Half-closed eyes were categorized as present or absent, through yes/no occurrences. Total duration of tail wagging was also assessed in seconds.

FIGURE 1 - A WHITE DORPER FEMALE SHEEP EXPRESSING HALF-CLOSED EYES AND BACKWARD EAR POSTURES DURING BRUSHING

In addition, we used an infrared thermometer gun model DT8530 to measure the temperature on 3 different body regions of the animals, per phase: withers (Tw), a region composed of hair, anal region (Ta), and the exterior part of the nose (Tn). The site at which we measured the wither temperature was different from the brushed site, to avoid any influence of mechanical stimulation. Different sites were taken as a way to assess which regions would accurately reflect the presumed positive state of the animals. The infrared thermometer presented a resolution of 0.1°C and it was equipped with a laser. Basic accuracy was considered to be ±2°C, according to the manufacturer's specifications. Accuracy was tested by measuring the temperature of a surface of a known temperature during the day of assessments, as described by Proctor and Carder (2015b). Temperatures were taken with the thermometer located approximately 10-15 cm from the sheep. The ambient temperature of each day of assessment was monitored using a portable digital

thermometer on the wall, and the experimenter B was responsible to check the temperature for each assessed animal.

### 2.2.6 Statistical analyses

Statistical analyses of behavioral and physiological variables were performed using the R program, Vienna, Austria (R DEVELOPMENT CORE TEAM, 2015). We fitted linear mixed models (LMMs), generalized linear mixed models (GLMMs), and marginal models, from nlme, lme4, and multgee packages, respectively. Because data included repeated measurements on the same animal, it was necessary to incorporate an intra-animal correlation to the analysis, by the inclusion of the random effect of animal for LMM and GLMM, or by modeling a correlation structure for marginal models. Phase, sex, and breed, in addition to the interactions among them, were considered as fixed effects.

Fixed effects and their interactions on behavioral and physiological variables were tested via likelihood ratio test for LMM and GLMM, and through the Wald test for marginal models. Interaction effects were not significant over any variable of interest at 5% level. For the estimation of parameters for LMM and GLMM, the maximum-likelihood method was used, whereas for marginal models, we applied the quasi-likelihood approach. For hypothesis testing and estimation purposes, a significance level of 5% and confidence interval at 95% were adopted.

For vocalization, it was not possible to adjust a model that satisfactorily described the responses. Instead, we used the nonparametric Cochran's Q test to test the hypothesis of equal proportions between the 3 phases, and McNemar test to compare the proportions in each pair of experimental phases. Both tests produced conflicting results, probably due to low and null frequencies of results.

The most frequent ear postures expressed by the animals were R, H, and B postures. The asymmetrical posture was only performed for longer by 1 male and 1 female Dorper sheep. Once it was not possible to estimate parameters for such posture, both animals were removed from the analyses. The predominant posture was modeled considering a multinomial distribution. To accommodate the correlation of observations in the same animal, a marginal model was fitted, by modeling the probability of postures through logit regression and specifying a correlation structure for intra-animal observations. The interpretation of results is based on odds ratio.

Odds ratio is given by the exponent of an estimated coefficient and it corresponds to, first, R and H, followed by B and H and B and R contrasts. All the fixed effects were significant for ear postures ($P<0.05$) and remained in the statistical model.

In relation to ear changes, 4 different methods were applied: Friedman test, model with normal distribution and inclusion of a random effect, model with Poisson distribution and inclusion of a correlation structure, and model with negative binomial distribution and inclusion of a random effect. None of the effects was significant at 5% level. Therefore, the results are reported descriptively.

Regarding the variable presence of half-closed eyes, we used the binomial distribution through GLMM, but the estimates did not converge. Thus, we applied the Cochran's Q test. When testing the hypothesis of equal proportions between phases, significant differences in the proportions of animals showing half-closed eye were observed across the 3 phases ($P<0.001$). Given the existence of differences, we executed the McNemar test.

For tail wagging, it was also not possible to adjust statistical models, as vocalization. Thus, the results for both variables are presented descriptively.

We conducted the Bartlett's test for homogeneity of variances, using the Bonferroni correction to compare the variability in Tw, Ta, and Tn. When adjusting models for Tw, Ta, and Tn, the variables were modeled via LMM and assuming a normal distribution. One female Dorper sheep was removed from the analyses for Tn because we found differing temperature values based on the analysis of residues. For Tw and Ta, all data were analyzed. It was found that for Tw and Tn, the effects were significant ($P<0.05$) and remained in the model. For Ta, no effect was significant ($P>0.05$).

## 2.3 RESULTS

During the brushing sessions, it was noted that 11 (9 Dorper and 2 White Dorper) sheep followed the experimenter A (median 3; minimum 1 to maximum 4 times), 7 (5 Dorper and 2 White Dorper) sheep leant against the brush (2; 2-3 times), 4 (3 Dorper and 1 White Dorper) sheep stretched the neck (3; 2-4 times); 5 (4 Dorper and 1 White Dorper sheep) kept still during brushing.

Infrequent vocalization was observed throughout the studied phases. Only 2 Dorper females and 1 male vocalized in the prebrushing phase. From these, only 1 female vocalized after brushing. During brushing, no vocalization was recorded.

Phase presented a significant effect on R and H contrasts (prebrushing vs. brushing: P=0.004); breed and sex were not significant. In the prebrushing phase, the probability of sheep showing a longer duration of R posture is estimated at 8.33 times the chance of showing the H posture (TABLE 1). In other words, sheep were more likely to express the R posture for longer rather than H posture in the prebrushing phase. Phase (brushing vs. postbrushing: P=0.04) and breed (P=0.01) were significant effects on B and H contrasts. The chance of sheep showing a longer duration of B than H posture during brushing was 3.85 the chance of sheep showing such postures after brushing (TABLE 1). In summary, it is more likely that sheep perform the B than the H position during brushing. The chance of Dorper sheep performing the B posture for longer duration than the H posture was approximately 10.52 times the chance of White Dorper sheep perform such postures (TABLE 1).

TABLE 1 - ODDS RATIO CALCULATED FOR RAISED UP (R) VERSUS HORIZONTAL (H), BACKWARD (B) VERSUS HORIZONTAL (H), AND BACKWARD (B) VERSUS RAISED UP (R) EAR POSTURES, IN 25 SHEEP, CONSIDERING THE CONTRASTS OF PHASE, SEX, AND BREED

| Ear postures | Contrasts | Odds ratio (OR) | OR Confidence Interval | P |
|---|---|---|---|---|
| R vs H | Pre vs brushing | 8.33 | 1.92; 33.33 | 0.004 |
| | Pre vs postbrushing | 3.33 | 0.88; 12.50 | 0.075 |
| | Brushing vs postbrushing | 0.41 | 0.08; 2.04 | 0.28 |
| | Male vs female | 0.47 | 0.12; 1.79 | 0.265 |
| | Dorper vs White Dorper | 0.93 | 0.18; 5.00 | 0.94 |
| B vs H | Pre vs brushing | 0.29 | 0.06; 1.35 | 0.115 |
| | Pre vs postbrushing | 1.14 | 0.33; 3.85 | 0.84 |
| | Brushing vs postbrushing | 3.85 | 1.08; 14.28 | 0.04 |
| | Male vs female | 3.57 | 0.76; 16.67 | 0.11 |
| | Dorper vs White Dorper | 10.52 | 1.77; 50.00 | 0.01 |
| B vs R | Pre vs brushing | 27.60 | 0.006; 43.21 | 0.0001 |
| | Pre vs postbrushing | 0.34 | 0.06; 1.92 | 0.225 |
| | Brushing vs postbrushing | 9.52 | 1.52; 50.00 | 0.02 |
| | Male vs female | 7.64 | 2.04; 28.57 | 0.003 |
| | Dorper vs White Dorper | 11.24 | 1.02; 125.00 | 0.05 |

Phase (prebrushing vs. brushing: P=0.0001; brushing vs. postbrushing: P=0.02), sex (P=0.003), and breed (P=0.05) were significant on B and R postures.

During brushing, in comparison with prebrushing, sheep were 27.60 times more likely to express the B posture rather than the R posture (TABLE 1). Likewise, when brushed, sheep were 9.52 times more likely to perform the B posture than the R posture, in comparison with the postbrushing phase (TABLE 1). The chance for male sheep to express the B posture rather than the R posture was 7.64 times the chance of female sheep express such postures (TABLE 1). Finally, Dorper sheep tended to express the B posture rather than the R posture for longer than White Dorper sheep (TABLE 1).

The estimated probabilities of male White Dorper and male Dorper sheep, respectively, expressing a longer duration of R posture in the prebrushing phase, were 0.68 and 0.62 (TABLE 2). During brushing, 0.71 was the estimated probability of Dorper sheep performing B as a frequent posture (TABLE 2). Similarly, 0.75 and 0.72 were the estimated probabilities of White Dorper male and female sheep, respectively, during brushing, perform the H posture for longer (TABLE 2); 0.49 and 0.26 were the probabilities for an increasing duration of such posture by Dorper male and female sheep, respectively, when brushed (TABLE 2). After brushing, 0.60 and 0.73 were the chances of White Dorper male and female sheep, in order, show the H posture for longer (TABLE 2).

TABLE 2 - ESTIMATED PROBABILITIES FOR PERFORMING RAISED UP (R), BACKWARD (B) AND HORIZONTAL (H) EAR POSTURES, ACCORDING TO PHASE, SEX AND BREED, IN 25 SHEEP

| Breed | Sex | Phase | R | B | H |
|---|---|---|---|---|---|
| Dorper | Male | Prebrushing | 0.62 | 0.07 | 0.31 |
| | | Brushing | 0.12 | 0.39 | 0.49 |
| | | Postbrushing | 0.34 | 0.11 | 0.55 |
| | Female | Prebrushing | 0.34 | 0.30 | 0.36 |
| | | Brushing | 0.03 | 0.71 | 0.26 |
| | | Postbrushing | 0.14 | 0.36 | 0.50 |
| White Dorper | Male | Prebrushing | 0.68 | 0.01 | 0.32 |
| | | Brushing | 0.20 | 0.06 | 0.75 |
| | | Postbrushing | 0.39 | 0.01 | 0.60 |
| | Female | Prebrushing | 0.48 | 0.04 | 0.48 |
| | | Brushing | 0.09 | 0.19 | 0.72 |
| | | Postbrushing | 0.22 | 0.05 | 0.73 |

Briefly, it is noted that the most frequent posture in the prebrushing phase was the R posture, whereas during and after brushing, the most observed postures were H and B, and H, respectively (FIGURE 2). From a total of 25 sheep, 15 and 14 animals performed the R and H positions for longer before brushing, respectively (FIGURE 2). When brushed, 17 and 15 sheep showed the B and H postures for longer duration, respectively (FIGURE 2). After brushing, 16 sheep performed mainly the H posture (FIGURE 2).

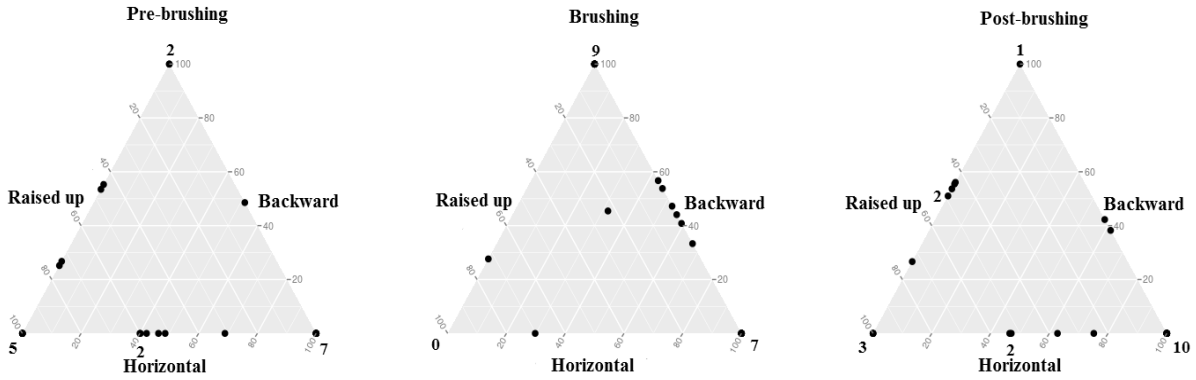


FIGURE 2 - TERNARY DIAGRAM PLOT WITH TOTAL DURATION DATA OF RAISED UP, BACKWARD AND HORIZONTAL EAR POSTURES FOR PREBRUSHING, BRUSHING, AND POSTBRUSHING PHASES, IN 25 DORPER AND WHITE DORPER SHEEP. THE MOST FREQUENT EAR POSTURES ARE REPRESENTED IN THE DIAGRAM. THE APEXES INDICATE THE TOTAL OF ANIMALS WHICH SPENT 100% OF THE TIME IN A GIVEN POSTURE. THE LOCATION OF CIRCLES IN THE DIAGRAM IS DETERMINED BY THE PROPORTION OF TIME EACH ANIMAL PERFORMED SPECIFIC POSTURES. NUMBERS ARE GIVEN WHEN 1 CIRCLE REPRESENTS MORE THAN 1 ANIMAL

Sheep showed a median number of ear-posture changes per phase equal to 10 (min 8; max 42), 6 (4; 26) and 7 (5; 39) times in the prebrushing, brushing, and postbrushing phases, respectively.

The McNemar test indicated significant differences in the prebrushing and brushing phases ($P \leq 0.001$), as well as at prebrushing and postbrushing phases ($P \leq 0.0001$) for the presence of half-closed eyes. During brushing animals tended to express half-closed eyes more than before the stimulus; after brushing, there was also a higher proportion of half-closed eyes than before brushing.

Regarding tail wagging, only 3 Dorper and 1 White Dorper male sheep expressed such behavior when brushed, with a median duration of 7.50 (4.38; 9.03) seconds. From these, only 1 Dorper sheep wagged the tail after brushing (total

duration: 1.18 seconds). No animal wagged the tail in the prebrushing and postbrushing phases.

Results for the test of homogeneity of variances per body region, through the comparison of standard deviation values, as well as the application of LMM to the mean temperatures per phase, are shown in TABLE 3. Significant differences between Tw and Tn (P=0.004) and Tw and Ta (P=0.00008) were noted in the prebrushing phase. During brushing, significant differences for Tw and Ta (P=0.00028) were also found. After brushing, significant differences between Tw and Tn (P=0.005) and Tw and Ta were noticed (P=0.00009). Based on the values, there is evidence of a higher variance for Tw in relation to Tn and Ta (TABLE 3).

TABLE 3 - WITHERS (TW), ANAL (TA) AND NASAL (TN) MEAN TEMPERATURES (ºC) MEASURED IN DIFFERENT EXPERIMENTAL PHASES, IN DORPER AND WHITE DORPER SHEEP

| Phases | Tw | Ta | Tn |
|---|---|---|---|
| Prebrushing | $28.43^{A*} \pm 3.27^{a}$ | $35.14^{A} \pm 1.44^{b}$ | $33.46^{A} \pm 1.83^{b}$ |
| Brushing | $28.74^{AB} \pm 3.06^{a}$ | $35.19^{A} \pm 1.44^{b}$ | $33.31^{A} \pm 2.08^{ab}$ |
| Postbrushing | $29.25^{B} \pm 3.14^{a}$ | $34.89^{A} \pm 1.39^{b}$ | $34.30^{B} \pm 1.39^{b}$ |

*DIFFERENT CAPITAL LETTERS IN COLUMNS REFER TO SIGNIFICANT DIFFERENCES BETWEEN MEAN TEMPERATURES PER PHASE, THROUGH LMM ($P < 0.05$); DIFFERENT LOWER CASE LETTERS IN LINES INDICATE SIGNIFICANT DIFFERENCES THROUGH THE TEST FOR HOMOGENEITY OF VARIANCE, ACCORDING TO EACH BODY REGION (P<0.05).

Furthermore, significant differences between the variances were observed for all assessed phases, when comparing Ta and Tn versus Tw, except during brushing, in which Tw and Tn were not significantly different. In the same way, between Ta and Tn, there were no statistical differences, that is, the variances of nasal and anal temperatures were similar (TABLE 3).

For the models adjusted using LMM, it was observed that the brushing effect on Tw was only significant in the contrast prebrushing and postbrushing (P=0.02; TABLE 3). In the postbrushing phase, Tw was, on average, 0.82ºC higher than in the prebrushing phase. For Tn, significant differences were found in the prebrushing and postbrushing phases as well as in brushing and postbrushing phases (TABLE 3). Nasal temperature after brushing was, on average, 0.99ºC higher than in the brushing phase (P=0.004; TABLE 3). In addition, in the postbrushing phase, Tn was, on average, 0.84ºC higher than in the prebrushing phase (P=0.01; TABLE 3). There

were no significant differences between prebrushing and brushing mean temperatures (P=0.67; TABLE 3).

## 2.4 DISCUSSION

The results show that ear postures and the presence of half- closed eyes, as well as withers and nasal temperatures, may be important indicators when assessing positive welfare in sheep. In addition, the studied sheep may have perceived brushing by a familiar observer as a positive stimulus. The behaviors following the observer, leaning against the brush with the head or neck, stretching the neck while brushed and standing still while brushed strongly suggest a positive perception of brushing by sheep. However, it is important to highlight that sheep were reluctant in the beginning of the adaptation sessions. This fact was also documented in a research of Pajor, Rushen and De Passille (2002) on dairy cows, in which brushing was more aversive than being given food or receiving no treatment, at least in the beginning of testing. Such facts highlight the importance of the adaptation period, so that the animals could overcome any eventual fear. An equally important issue to be addressed is the individual variation, as 6 sheep did not adapt to the procedure during the adaptation period under the given time frame.

Vocalization was not observed during brushing. Proctor and Carder (2015a,b) argued that stroking may elicit a positive, low arousal emotional state, and we suggest that the description may be extrapolated to brushing as well. In a study on familiarity and predictability of events in Romane sheep, Greiveldinger, Veissier and Boissy (2007) reported that a sudden event might have been perceived as negative by the animals, which vocalized more; in contrast, sheep submitted to the regular appearance of a sudden event, providing predictability, showed an increase in food consumption and a decrease in vocalization. Such results suggest that vocalization may be considered an indicator of negative welfare. This may be related to the low occurrence of vocalization observed, considering that our experiment was designed to expose animals to a low arousal, positive stimulus. Further studies may compare behavioral responses of sheep toward presumed positive situations with special attention to vocalization. In addition, other parameters of vocalization, associated with more sophisticated measures to distinguish positive and negative

events, may also be included to better understand its relation to affective states in animals.

We observed a higher duration of raised up ears before brushing, horizontal and backward ears during brushing, and horizontal ears after brushing. Fridlund (1991) reported that there is homology between the muscles involved in both lowering eyebrows in humans and ear mobility in animals. Eyebrow movements, including eyebrow lowering, are used in a variety of negative emotions, such as fear, sadness, and anger (EKMAN, 1979). This finding may be relevant for ear postures in animals, suggesting that negative situations would trigger the expression of raised up postures, whereas positive situations would reflect in expressions of non-erect ear postures (BOISSY et al., 2011), as horizontal and backward postures. We noted that raised up ears differed significantly from backward postures when prebrushing and postbrushing phases were compared. Raised up ear postures in the anticipation phase may be expressed in response to increased attention and may occur in anticipatory phases of positive events (SPRUIJT; VAN DEN BOS; PIJLMAN, 2001). We believe that the studied animals predicted a positive situation and, consequently, increased their attention toward it. During brushing, a higher duration of horizontal was noted. Reefmann, Wechsler and Gygax (2009) observed that Swiss white alpine and Lacaune sheep groomed by a familiar observer presented increased proportions of horizontal ears. Coulon et al. (2015) also reported a high proportion of hanging and horizontal ear positions in Romane sheep during stroking, and suggested that the physical contact may have produced a calming effect on the animals. In addition, we noted that, when brushed, sheep showed the backward ear posture for longer, and such fact is in accordance with literature data for animals submitted to presumed positive events, as stroking (PROCTOR; CARDER, 2014) and hay feeding (REEFMANN et al., 2009a), although it was also associated with unfamiliar and unpleasant uncontrollable situations in Romane sheep (BOISSY et al., 2011). Differing results might be due to the fact that ears can hang backward (passively and relaxed) or be tensed and actively directed backward, and it might depend on the angle of the video camera or the head position. In our study, we believe that the backward posture may have reflected a positive experience as result of tactile stimulation. After brushing, horizontal ears were expressed for longer. As horizontal ears seem to reflect a positive state, we believe that the postbrushing phase was

characterized by the elicitation of an appeasing, relaxing state, suggesting that brushing had a carryover effect on sheep behaviors.

The occurrence of specific ear postures was statistically significant when sex and breed were considered. We observed that male sheep tended to show the backward posture for longer rather than the raised up posture, when compared to female sheep. As, in our work, a longer expression of backward posture may be associated with the perception of a presumed positive event and, in general, male sheep seem to be less fearful than female sheep (BOISSY et al., 2005), the results support the idea that brushing had a calming effect mainly on males. As our results presented behavioral differences between males and females, future research may compare behavioral indicators of emotional states with focus on sex effects. The fact that Dorper and White Dorper sheep expressed significantly different ear postures for backward versus horizontal ears and backward versus raised up ears is an interesting result. Dorper and White Dorper are similar breeds, except regarding color and pigmentation, and the same breed standards are applied for both breeds (MILNE, 2000). No scientific finding on Dorper and White Dorper differences in behavior was found. However, it is noteworthy saying that selection for hair color resulted in important differences in temperament in some species of animals (KEELER, 1968). This effect of subtle sheep breed differences on ear postures presents a surprisingly high significance for genetic lineage, which warrants further research and careful comparisons across results from different breeds.

Different values for ear changes were observed for the assessed phases, with no significant result. Several studies report that increases in ear-posture changes reflect negative experiences in sheep submitted to different negative events (REEFMANN et al., 2009a; REEFMANN; WECHSLER; GYGAX, 2009). Reefmann et al. (2012), in a study about mood modulation in Swiss white alpine and Lacaune sheep, found that the number of ear-posture changes was high during separation (negative situation) and low during grooming (positive situation). We did not compare brushing to stimuli of different valence, which could promote significant differences. Furthermore, we believe that the animals may have experienced an appeasing state regardless of the phase, which possibly explains the absence of differences in ear movements among phases.

We noted significant differences in the prebrushing phase in relation to brushing and postbrushing for half-closed eyes. The animals tended to express half-

closed eyes mainly during brushing and after brushing. Some research on cows has revealed interesting results on the association between emotional states and eye aperture or visible eye white (SANDEM; BRAASTAD; BOE, 2002). In our study, most of the animals showed half-closed eyes in brushing and postbrushing phases, which may be interpreted as a sign of relaxation and pleasantness for the consummatory and postconsummatory phases of a positive situation, respectively. Reefmann, Wechsler and Gygax (2009) observed that sheep eyes were less wide open when they were assessed in a feeding area. Even lower eye aperture was found while the animals were groomed by a familiar experimenter, when compared to the situation of being separated from group members. In cows, a large percentage of visible eye white was noted in food-deprived animals, facing an important negative situation, and a decrease in animals that received food (SANDEM; BRAASTAD; BOE, 2002). For the positive situation, the animals performed the consummatory face, characterized by the presence of half-closed eyes, usually observed while animals eat, ruminate, or rest (SANDEM; BRAASTAD; BOE, 2002). The authors hypothesize that a high percentage of visible eye white is a sign of frustration or negative experience. In a recent research (PROCTOR; CARDER, 2015a), a significant decrease in the percentage of visible eye white during stroking was verified, in comparison with prestroking and poststroking phases. As assessments of eye aperture in animals require sophisticated equipment, we suggest that the presence of half-closed eyes may be a useful indicator of positive emotional valence.

In relation to tail wagging, we observed that only male sheep wagged their tails, during brushing. It is interesting to note that, during the adaptation sessions, 3 female Dorper sheep also expressed such behaviors. The literature does not provide concrete associations between sheep tail movements and emotional states. In a study on behavioral and postural indicators of pain associated with different husbandry practices in lambs, Grant (2004) observed that tail wagging was related to teat seeking and suckling activities. The author claimed that tail wagging was a poor differentiator between groups. In dogs, tail wagging is related to positive emotional states (FATJÓ et al., 2007). In pigs, Reimert et al. (2013) noted that tail movements occurred more often during a rewarding event, although other authors have documented such behavior in negative situations (ZONDERLAND et al., 2009). These controversial literature findings, with no direct association of tail wagging with positive or negative situations, point out to the need for further research.

Regarding body surface temperatures, few studies explored the relation between temperature taken from different body regions alone or in combination and emotional states in animals. This is the first study to assess temperatures taken from different body regions and relate them to emotional states in sheep. Not all the temperatures may accurately reflect the physiological state of the animal. For example, withers and nasal temperatures differed between prebrushing and postbrushing phases, with an increase in temperature after brushing; an increase in nasal temperature was also observed between the brushing and postbrushing phases. The significant differences, mainly before and after brushing reflected by Tw and Tn, may be associated to peripheral vasodilation and rise of superficial temperature after brushing. Such response may be related to positive emotions. Evidences of a more appeasing state after brushing were also supported by the assessed behaviors, as ear postures and half-closed eyes. Therefore, the results show that withers and nasal temperatures may have reflected similar physiological states during and after brushing. Different body regions may reflect similar responses when the same stimulus is assessed; for example, dorsal surface and eye temperatures increased in response to removal of velvet antler in elk and reindeer (COOK et al., 2005), assessed through infrared thermography. However, no significant differences were observed in the anal region. Such finding may indicate that such region is not as responsive to changes in temperature for peripheral vasodilation, as withers and nasal regions.

In general, peripheral temperatures in mammals seem to decrease in negative situations. When an individual faces a potentially stressful stimulus, a sympathetically mediated vigorous vasoconstriction initiates; consequently, the skin temperature decreases. In Romney-cross sheep, a reduced ear-pinna temperature and an increased vaginal temperature were observed in situations that elicited stress, as isolation and prolonged exercise (LOWE et al., 2005). Reefmann et al. (2009b), in a study on Swiss white alpine and Lacaune sheep, verified that body surface temperature taken from the sheep's midside increased during anticipation of feeding in comparison with the feeding phase, but did not differ within treatments, that is, if sheep received familiar standard feed, unpalatable wooden pellets, or enriched feed. The authors justified the results by the fact that the negative situation caused by unpalatable wooden pellets might not have been intense enough to change body surface temperatures. Similarly, absolute body surface temperature did not differ

significantly when sheep were separated from conspecifics (negative valence), stood in the feeding area (intermediate valence) or groomed (positive valence) (REEFMANN; WECHSLER; GYGAX, 2009). Such results suggest that body surface temperatures may be similar even in events differing in valence.

Regarding nasal temperatures, Nakayama et al. (2005) applied an infrared thermographic system to assess changes in the facial skin temperatures in rhesus monkeys. The animals were exposed to a potentially threatening stimulus and tested during 3 successive 3-minute phases, comprising prestimulation, stimulation, and poststimulation. Significant decreases in the nasal skin temperature occurred during the stimulation phase. In cows, the mean nasal temperature dropped significantly during stroking, when compared with mean temperatures before and after the stimulus (PROCTOR; CARDER, 2015b). As the study of nasal temperatures is quite recent, further research is necessary to determine if both positive and negative events result in a drop in nasal temperature. Our results confirm other findings from the literature which show that temperature may increase or decrease depending on the species, body regions, and valence or arousal of the stimuli involved. Because of the contradictory data in relation to temperature and emotional states, our results must be interpreted with caution. However, given the significant differences observed in the present research, we suggest that withers and nasal temperatures may be valuable tools to be explored in further studies that aim to assess emotional states in animals.

## 2.5 CONCLUSIONS

Ear postures and presence of half-closed eyes provided potential evidence that the studied animals experienced a situation that elicited positive emotions. Tail wagging may also be an important indicator of affective states in sheep, which require further research. Withers and nasal temperatures may be promising indicators to study emotions, as they seem to increase in response to positive stimuli. We also conclude, in a serendipitous manner, that breed characteristics may be a highly significant factor to interpret ear-posture data and warrant further studies. Our work has contributed to the understanding of positive emotions in animals, especially advancing knowledge regarding potential objective indicators, which will hopefully

enable us to improve animal welfare and promote human-animal interactions toward a greater respect for their sentient capacities.

## 2.6 ACKNOWLEDGMENTS

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# 3. INDUCING POSITIVE EMOTIONS: BEHAVIOURAL AND CARDIAC RESPONSES TO HUMAN AND BRUSHING IN EWES SELECTED FOR HIGH VS LOW SOCIAL REACTIVITY

## RESUMO

Avaliou-se a percepção de 38 ovelhas da raça Romane em relação à presença humana (HUM) ou à escovação por um observador familiar (BRU). As ovelhas pertenciam a duas linhagens genéticas: mais (R+) ou menos (R-) reativa. O procedimento foi dividido em três fases: antes (2,5 min), durante (3,0 min) e após (2,5 min) HUM ou BRU. Após quatro semanas de adaptação, as expressões comportamentais do animal teste foram registradas em vídeo e sua atividade cardíaca foi aferida por meio de um monitor de frequência cardíaca (FC). Posturas corporais, orientação da cabeça, mudanças e posturas de orelhas, grau de abertura do olho, movimentação da cauda e comportamento alimentar foram analisados, e os parâmetros da variabilidade da frequência cardíaca (RMSSD, RMSSD/SDNN e LF/HF) foram estimados. Os dados foram analisados utilizando modelos lineares com diferentes estruturas de correlação. Os modelos consideraram tratamento, linhagem genética e fase como efeitos fixos, incluindo interações. Ovelhas HUM apresentaram menos mudanças de postura corporal antes do procedimento do que ovelhas BRU, enquanto que durante o procedimento observou-se o oposto ($P<0,05$). Durante o procedimento, ovelhas HUM apresentaram mais mudanças na orientação da cabeça do que ovelhas BRU ($P<0,01$). Além disso, para ovelhas R+, animais do tratamento HUM apresentaram mais mudanças na orientação da cabeça ($P<0,01$). Durante o procedimento, menos mudanças de posturas de orelhas e maior proporção de tempo de olhos fechados e semicerrados foram encontrados para ovelhas BRU ($P<0,01$). Entre ovelhas R+, animais do tratamento HUM expressaram posturas levantada e assimétrica por mais tempo ($P<0,05$). Ovelhas BRU também movimentaram suas caudas por mais tempo do que as ovelhas HUM principalmente durante e após o procedimento ($P<0,01$). Entre ovelhas R+, animais do tratamento BRU passaram mais tempo comendo ou ruminando do que ovelhas HUM ($P<0,01$). A FC durante e após os procedimentos foi menor do que antes, nos grupos escovado e não ($P<0,01$). Não foram observadas diferenças significativas no indicador RMSSD, mas a razão RMSSD/SDNN durante o procedimento foi maior do que antes e depois ($P<0,05$). A razão RMSSD/SDNN em ovelhas R- foi maior do que em ovelhas R+ ($P<0,05$), revelando uma maior ativação do sistema parassimpático em ovelhas R- em resposta aos procedimentos. Maiores valores de LF/HF foram encontrados para ovelhas BRU após o procedimento, quando comparados a ovelhas HUM ($P<0,05$). Os resultados sugerem que ambos os tratamentos induziram um estado relaxante nos animais, mais pronunciado para o grupo escovado. Curiosamente, agitação na fase antecipatória dos tratamentos foi observada principalmente em animais escovados. Além disso, diferenças entre animais R+ e R- sugerem que a reatividade pode modular as respostas das ovelhas para estímulos positivos presumidos.

Palavras-chave: Estimulação tátil. Indicadores positivos. Manejo humano. Ovinos. Reatividade emocional. Respostas autonômicas.

## ABSTRACT

We assessed the perception of 38 Romane ewes towards the mere presence of a familiar experimenter (HUM) or brushing by a familiar experimenter (BRU). The ewes belonged to two genetic lines: high (R+) or low (R-) reactive. The procedure was divided in three phases: before (2.5 min), during (3.0 min) and after (2.5 min) HUM or BRU. After four weeks of adaptation, the behavioural expressions of the test animal were video recorded and its cardiac activity was registered through a heart rate (HR) monitor. Body postures, head orientation, ear changes and postures, eye aperture, tail wagging and feeding behaviour were analyzed, and heart rate variability parameters (RMSSD, RMSSD/SDNN and LF/HF ratios) were estimated. Data were analyzed using linear models with different correlation structures. The models considered treatment, genetic line and phase as fixed effects, including their interactions. Ewes in the HUM treatment showed less body posture changes before the procedure than BRU ewes, whereas during the procedure, the opposite was observed ($P<0.05$). During the procedure, HUM ewes showed higher number of head orientation changes than BRU sheep ($P<0.01$). In addition, for R+ ewes, HUM sheep showed more head orientation changes ($P<0.01$). During the procedure, a lower number of ear changes and a higher proportion of closed and half-closed eyes were found for BRU sheep ($P<0.01$). Among R+ sheep, HUM sheep showed raised up and asymmetric ear postures for longer ($P<0.05$). Ewes in the BRU treatment also wagged their tails for longer that HUM sheep mainly during and after the procedure ($P<0.01$). Among R+ sheep, BRU ewes spent more time eating or ruminating than HUM ewes ($P<0.01$). The HR during and after the procedure was lower than before the procedure, whether brushed or not ($P<0.01$). No significant differences in RMSSD were noted, but the RMSSD/SDNN ratio during the procedure was higher than before and after ($P<0.05$). The RMSSD/SDNN ratio in R- ewes was higher than in R+ ewes ($P<0.05$), revealing a stronger activation of the parasympathetic system in R- sheep in response to the procedures. Higher LF/HF values were found for BRU sheep after the procedure, when compared with HUM sheep ($P<0.05$). The results suggest that both treatments induced a relaxing state in sheep, more pronounced for the brushing treatment. Interestingly an anticipatory agitation before the treatment was observed mainly in brushed sheep. Furthermore, differences between R+ and R- suggest that reactivity might modulate sheep responses to assumed positive affective stimuli.

Keywords: Tactile stimulation. Positive indicators. Human handling. Sheep. Emotional reactivity. Autonomic responses.

## 3.1 INTRODUCTION

During the last decade, the study of farm animal sentience and welfare has focused on promoting positive emotional states (BOISSY et al., 2007; YATES; MAIN, 2008; MELLOR, 2015), representing a relevant additional approach to the historical research centered on understanding negative welfare. Pleasure, contentment and playfulness may be cited as some positive emotional states in animals (MELLOR, 2012). According to Boissy et al. (2007), positive affective states may be classified in three temporal categories in relation to the stimulus: (i) past for post-consummatory satisfaction, (ii) present with enjoyment of a given pleasant situation and (iii) future for positive expectation. Presumed positive situations, as play, feeding and gentle contact, such as stroking, grooming and brushing, have been reported to be perceived positively by ruminants, through behavioural and physiological inferences.

Ear postures and changes, relative eye aperture and tail moves and postures have been commonly assessed in studies of emotional states in animals (REEFMANN et al., 2009; REEFMANN; WECHSLER; GYGAX, 2009; REIMERT et al., 2013; COULON et al., 2015). In addition to behavioural indicators, the influence of emotional states on the autonomic nervous system has been explored in different animals (RIETMANN et al., 2004; STUBSJØEN et al., 2009; BRIEFER; TETTAMANTI; MCELLIGOTT, 2015). Heart rate reflects the autonomic tone, understood as the interaction between sympathetic and parasympathetic activities. Heart rate variability (HRV), a non-invasive measure of the autonomic nervous system, has been used as a parameter for measuring emotional states in animals, including the root mean square of successive R–R interval differences (RMSSD), reflecting alterations in the parasympathetic system, the standard deviation of all inter-beat intervals (SDNN), which represents vagal and sympathetic influences on heart rate, and the RMSSD/SDNN ratio. In addition, the LF/HF ratio, a frequency domain measure, reflects a sympathovagal balance, being an increase in the LF/HF ratio interpreted as an accentuation of sympathetic activity (VON BORELL et al., 2007).

Behavioural and cardiac reactions in animals in response to stressful stimuli and environmental challenges, known as emotional reactivity, have an important effect on how individuals interact with and respond to the environment. Emotional reactivity seems to be influenced by genetics in livestock species (BOISSY et al.,

2005; MIGSON-GRASTEAU et al., 2005). Therefore, we aimed to study the perception of sheep to the exposition to a motionless familiar human, and to brushing, using behavioural and cardiac indicators. We also described the behavioural and cardiac responses linked to anticipatory (pre-procedure), consummatory (procedure) and post-consummatory (post-procedure) phases of the proposed stimuli. As sheep may perceive gentle handling by a familiar human as pleasant (REEFMANN; WECHSLER; GYGAX, 2009; COULON et al., 2015), we hypothesized that brushing triggers more anticipatory responses before, accompanied by sympathetic activation, and during and after brushing, ewes express more relaxing, calming responses, followed by parasympathetic activation, than ewes submitted to the motionless human. In addition, as in sheep behavioural reactivity to social separation is reported to be highly heritable (BOISSY et al., 2005; HAZARD et al., 2014), the altering effects of the social reactivity on the responses to a familiar human and to brushing were also tested using sheep from two different genetic lines, selected for behavioural reactivity to social separation (high (R+) and low (R-) reactivity, unpublished data). As highly reactive animals are known to be more frequently involved in affiliative interactions (SCHÜRCH; ROTHENBERGER; HEG, 2010), we expected a stronger alteration of positive markers in comparison to lowly reactive sheep.

## 3.2 MATERIAL AND METHODS

### 3.2.1 Animals and management

The experiments were carried out at INRA experimental farm La Fage (Roquefort, South of France). Thirty-nine 15-month-old Romane female sheep were used. Sheep were born on the farm where the research was conducted and they were reared as one large group at pasture before and after the experiments. They belonged to two divergent genetic lines, currently selected according to their behavioural reactivity towards a temporary social separation, early assessed in two behavioural tests: arena and corridor tests (for the testing method, see LIGOUT et al., 2011). More reactive sheep (R+) and less reactive sheep (R-) were selected by bouts of high bleats; R+ sheep were also characterized by higher locomotor activity and lower time spent in vigilance postures than R- sheep (unpublished data). Since

2010, each year, female and male lambs from 250 dams are submitted to the behavioural tests just after weaning. Using pedigree and behavioural phenotypes, individual estimated breeding values (EBV) were estimated for each lamb using a linear mixed model and the BLUP method (Best Linear Unbiased Prediction) on ASREML software. Each year, extreme animals were chosen according to their high or low EBV for social reactivity and used to produce the next generation of animals. For the present experiment, the 39 female sheep with low (R-, n=21) and high EBV (R+, n=18) were chosen as extreme animals and belonged to the first generation of selection. The approval of the study by an Animal Ethics Committee was not required in France. However, the study meets the requirements of the International Society of Applied Ethology ethical guidelines (SHERWIN et al., 2003). After the experimental period, the animals went back to pasture and remained on the farm of origin.

One week before the beginning of the treatments, the animals were taken from the pasture and familiarized to indoor conditions with straw bedding. Sheep were separated in groups, considering the treatment (presence of a familiar experimenter (HUM) or brushing by a familiar experimenter (BRU)) and genetic line (R+ and R-). The division of the groups (R+ HUM; R+ BRU; R- HUM; R- BRU) was repeated in order to avoid confounding the experimental effects with a putative group effect. Thus, for each line, the animals were randomly assigned to eight groups: Group 1: R+ HUM (n=5); Group 2: R- BRU (n=6); Group 3: R- HUM (n=5) and Group 4: R+ BRU (n=4), on the right side of the hangar; Group 5: R- BRU (n=5); Group 6: R+ HUM (n=4); Group 7: R+ BRU (n=5) and Group 8: R- HUM (n=5), on the left side of the hangar. In total, there were 20 sheep belonging to the BRU treatment (9 R+ and 11 R-) and 19 to the HUM treatment (9 R+ and 10 R-). Water was available *ad libitum* and the feeder was filled with hay twice a day, at 06:00 and 18:00 h. Wooden bars separating the groups allowed partial to no visual interactions with animals belonging to the other treatments; sheep could hear the animals from the other pens.

### 3.2.2 Adaptation period

The adaptation period to the treatments lasted four weeks. Each animal was handled twice a day, over six consecutive days per week. At the same time, animals were exposed to the equipment to measure behaviour (two cameras positioned on two monopods and one camcorder) and heart rate (an elastic belt which simulated

the heart rate monitor which was put on the animals on the testing days), one barrier to separate the test animal and group members, and a stopwatch. Sheep were also trained to the presence of two experimenters: 1) a female experimenter (experimenter 1), who held a camcorder and a stopwatch, and was responsible for performing the HUM and BRU treatments, and 2) a male experimenter (experimenter 2), who was responsible for bedding and food delivery, as well as for fitting elastic belts to the animals.

During the adaptation period, experimenter 1 sat on a white bench, located in the same position during all sessions. Four groups of animals were assessed in the morning (08:30 to 12:30) and the other four groups in the afternoon (from 13:30 to 17:30). All groups were assessed respecting a fixed order and period which was maintained until the testing days. The elastic belts were put on the animals respecting the time of testing and the size of each animal. There were 20 belts in total, thus 20 sheep were simultaneously equipped in the morning and the other 19 in the afternoon. Each animal was assessed during 6 min, comprising three phases: Phase 1 - Pre-procedure: 2 min before the entrance of experimenter 1 in the testing area; experimenter 1 was beside the metal grid, outside the testing area, and positioned approximately 40 cm from the test animal; Phase 2 - During the procedure: 2 min with the animal, experimenter 1 entered the pen with the bench, brushed the animal or only stood still, and Phase 3 - Post-procedure: 2 min after the stimulus, the procedure was similar to the first phase.

Animals were assessed individually and as a group according to the week. On the first week of adaptation, the animals were assessed as a group and they were kept closer to experimenter 1, as she put a barrier occupying two-third area of the pen. At this moment, experimenter 1 was motionless and only observed the animals, but did not try any physical contact. All the animals received the same treatment, i.e., they were submitted to the mere presence of the experimenter. During the second week, the animals were also assessed in groups and they were also submitted to HUM or BRU treatments. Sheep belonging to the BRU group had the first contact with a 15 cm x 7 cm bristle (1 cm length) plastic-handled brush. The brush was chosen since Romane sheep have short wool, so that the bristles moved freely through it. Animals were brushed randomly. Speed of brushing ranged between 20 to 40 brushing/strokes per minute and the standardized body regions to be brushed were ventral neck, lateral chest, withers and belly. Such regions were selected based

on preferred regions to be stroked by cattle (SCHMIED; BOIVIN; WAIBLINGER, 2008) and sheep (TAMIOSO et al., 2017). For the HUM group, experimenter 1 did not try either eye or physical contact with the animals. During the third and fourth weeks, in addition to the respective treatments, each test animal was adapted to being separated from the group members by a metal barrier (1.80 m length and 1.24 m height), which allowed visual and olfactory social contact (FIGURE 3). During all the adaptation period, experimenter 1 maintained behavioural monitoring of each animal regarding their acceptance or not towards her and the treatment. Some behaviours that were used to select animals which would participate in the study and characterized acceptance were observed: following the observer, leaning against the brush with the head or neck and neck stretching, based on findings by Westerath, Gygax and Hillman (2014) on the perception of calves to brushing. We observed that 38 ewes approached the experimenter and 27 ewes stayed beside the experimenter; 32 ewes explored the experimenter with head, muzzle or hooves (number of occurrences per animal: median 3; minimum 1- maximum 4), 29 ewes touched or pushed the experimenter's knees with the head (2;1-4), 12 followed the experimenter (2;1-3), 12 leant against the brush (for BRU only) (2;1-4) and nine BRU ewes stretched the neck while brushed (2;1-3). In the end, one sheep (treatment HUM, line R+) was removed from the study due to no adaptation to the testing conditions, i.e. non-acceptance of the experimenter and treatments as expressed by avoidance behaviour (escape from the pen), there remaining 38 ewes in total.

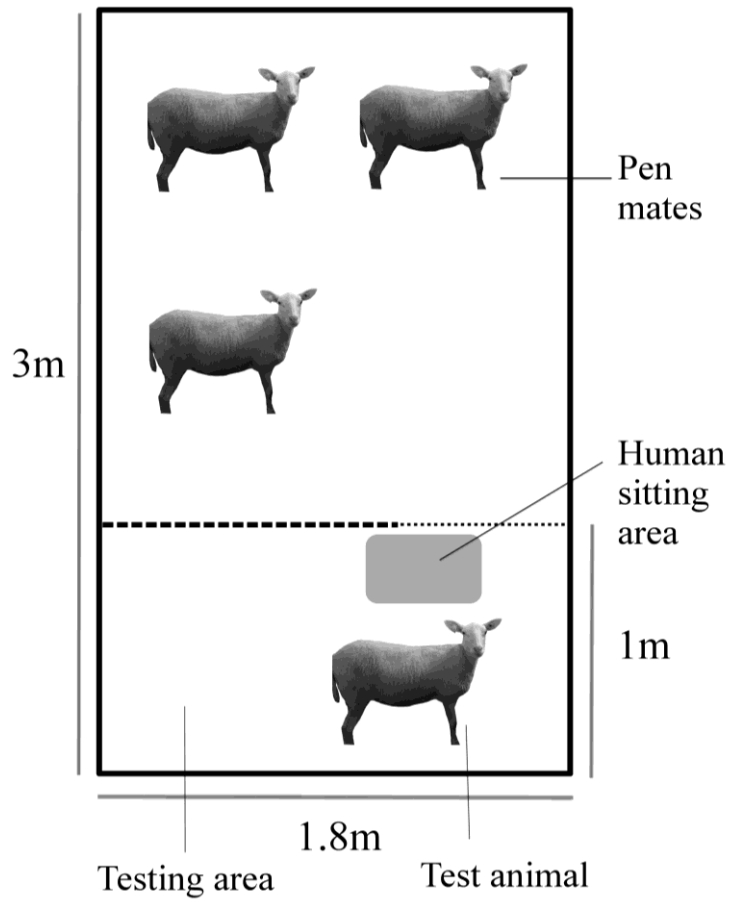


FIGURE 3 - DIAGRAM OF THE HOME PEN WHERE 17 HIGHLY AND 21 LOWLY REACTIVE EWES WERE SUBMITTED TO HUMAN PRESENCE AND BRUSHING, WITH DIMENSIONS (THICK DOTTED LINES: METAL BARRIER SEPARATING THE TEST ANIMAL AND PEN MATES; FINE DOTTED LINES: OPENING THORUGH WHICH THE TEST ANIMAL AND EXPERIMENTER 1 HAD ACCESS TO THE TESTING AREA; THE BENCH INDICATES THE POSITION OF EXPERIMENTER 1 DURING EACH PROCEDURE)

### 3.2.3 Experimental design

Animals were tested during four consecutive days two days after the adaptation period was finished. The animals were assessed once and individually, through the metal grid separating the test animal from pen mates. The experimental session considered the same three phases of the adaptation period, with minor differences in time of assessment. In total, the testing period took 8 min to be performed, divided in: Phase 1 - Pre-procedure: 2 min 30 s; Phase 2 – During the procedure: 3 min; and Phase 3 - Post-procedure: 2 min 30 s.

Brushing was performed as described during the adaptation period. Brushing initiated immediately after the entrance of experimenter 1 in the pen, where the test animal was separated from the group members. Then, experimenter 1 started the

stopwatch and the camcorder. Experimenter 2 was concealed in an area ranging from 5 to 20 m far from the animals, and he quietly took notes on sheep identification and order, recorded the cardiac information into a software, as well as monitored the cameras. He also replaced the heart rate monitors from one group to the other, after finishing each assessment. The monitors consisted of a 100 g chest belt with connection of three integrated electrodes and a telemetric wireless transmitter (EMKA Technologies, Paris, France, EMKAPack 4G). Two electrodes were put on the left side of the chest and one electrode was put near the caudal angle of the scapula and close to the sternum. The heart rate belt was fitted on the animal following the instructions provided by the manufacturer. It took approximately 3 min to put the equipment on each animal. A transmission gel was applied under the electrodes in order to reduce skin resistance and optimize conductivity. Three days before the tests, all the animals were shaved in the area where the heart rate equipment, as well as the electrodes, were placed by a stockperson who did not participate in the study.

Four heart rate monitors were used at the same time. They were placed on the animals according to the order the groups were assessed during the adaptation period, and kept until the end of the testing of the last animal of the group. Thus, the equipment recorded information during a period which ranged from 10 to 50 min, approximately. The period to assess each animal ranged from 10 to 15 min. When the assessment of the last fourth animal was finished, experimenter 2 entered the pen and put the monitors on the next four animals to be tested. Experimenter 1 waited five minutes after experimenter 2 left the pen to start the test. The information concerning each relevant period of evaluation was selected for analyses.

### 3.2.4 Behavioural and cardiac indicators

Animals were video-recorded during the observation periods. Body postures, head orientation, ear changes and postures, eye aperture, tail wagging and feeding behaviour were assessed. The experimental ethogram with all behavioural variables is presented in TABLE 4.

TABLE 4 - EXPERIMENTAL ETHOGRAM CONTAINING THE BEHAVIOURAL VARIABLES OBSERVED DURING TESTING SESSIONS

| Behaviour | Categories | Definition |
|---|---|---|
| Body postures | Lying | Sheep with the belly and flanks in contact with the floor |
| | Standing, still | Sheep standing still with the four hooves in contact with the floor |
| | Standing, moving | Sheep in movement with the four hooves in contact with the floor |
| Head orientations | Head to pen mates | Head oriented toward the animals from the group |
| | Head to the experimenter | Head oriented toward the female experimenter |
| | Head to the pen walls or the metal grid | Head oriented toward the walls of the pen or the barrier that separated the test animal from pen mates |
| | Head to the floor | Head oriented toward the floor of the pen |
| | Other | Head oriented toward any other stimuli that do not contemplate the aforementioned; example: no specific openness in relation to the external environment, no apparent willingness to interact and involve with outside |
| Ear postures[1] | Raised up | The two ears are ahead or aligned |
| | Horizontal | The two ears are in the frontal plane |
| | Backwards | The two ears are behind the frontal plane |
| | Asymmetric | The two ears are in distinct position in relation to the frontal plane |
| | Non visible | Not possible to clearly visualize the ears from the video recordings |
| Eye aperture | Eyes open | Eyes wide-open, no visible eyelids or eyelids visible up to the middle portion of the eye |
| | Eyes half-closed | Upper eyelids in the middle portion of the eye |
| | Eyes closed | The upper eyelid is totally in contact with the lower eyelid |
| | No visible | Not possible to clearly visualize the eye from the video recordings |
| Tail wagging | Tail wagging | Tail swinging up and down or side to side |
| | Tail not wagging | Tail still; not swinging in any direction |
| Feeding behaviour | Eating | Lowering the head with muzzle and consuming food |
| | Ruminating | Regurgitation, chewing and swallowing of previously eaten food |
| | Not eating or ruminating | Sheep not eating or ruminating |

[1]ADAPTED FROM BOISSY et al. (2011)

The cardiac variables assessed were mean heart rate value (HR); root mean square of successive NN interval differences (RMSSD), which reflects an estimate of parasympathetic regulation; standard deviation of NN intervals (SDNN), a measure of both sympathetic and parasympathetic activities; and the RMSSD/SDNN ratio. We also recorded the ratio between low-frequency (LF) power, which reflects both sympathetic and parasympathetic activities, and high-frequency (HF) power, which reflects the parasympathetic activity.

### 3.2.5 Data analysis

Behaviour was analyzed through video recordings and coded in terms of total duration and number of changes using the Experimenter XT version 11.5 (Noldus Information Technology, Wageningen, The Netherlands). As several behavioural indicators were tested, we chose to present the results for the data analyzed in two different ways: number of variations and proportion of time. Cardiac variables were analyzed using EMKA ECGauto version 3.3.0.30 (EMKA Technologies, Paris, France), a software that simultaneously processes values for time and frequency domain variables based on the inter-beat (RR) intervals, through the function “ECG Analysis”. In order to select the segments for frequency domain analyses, fixed limits were set, as 0.04 to 0.15 Hz for LF and 0.15 to 1 Hz for HF. Mean HR (bpm) was calculated using the formula HR=60000/RR interval; RMSSD in ms; SDNN in ms; RMSSD/SDNN ratio and LF/HF ratio.

Statistical analyses were performed using the R program, version 3.2.2. As data included repeated measurements on the same animal, it was necessary to incorporate an intra-animal correlation to the analyses. Treatment (HUM or BRU), genetic line (R+ or R-) and phase (pre-, during and post-procedure), and the interactions amongst them were considered as fixed effects. Estimates given for a single effect or interactions were obtained considering the mean effects. When phase was significant over the variables of interest, an approach for multiple comparisons was used, in order to reach the set level of significance between the three contrasts (pre- vs during, during vs post- and pre- vs post-procedure). For hypothesis testing, the significance level of 5% and confidence interval at 95% were adopted. For the behavioural variables, data were analyzed using generalized linear models, with estimation via generalized estimating equations. The functional relationship between the mean response and the effects of other experimental factors were defined by the logarithmic function for the variables related to the number of variations, as body postures, head orientation and ear posture changes, and by the logit function in case of variables assessed as proportion of time, such as ear postures, eye aperture, tail wagging and feeding behaviour. For eye aperture, closed and half-closed eyes were analyzed together, due to the small number of animals with closed eyes. For ear postures, two different analyses were performed, from the most frequent positions in each phase: raised up, horizontal and asymmetric postures. The first analysis

considered the proportion of time spent on the raised up ear, calculated as: p = (raised up)/ (raised up + horizontal), and the second considered the proportion of time spent on asymmetric ear: p = (raised up + asymmetric)/ (raised up + horizontal + asymmetric). For feeding behaviour, we considered the proportion of time the animal spent eating or ruminating, analyzed together.

We also fitted linear models with normal errors for cardiac variables. Significances of fixed effects were estimated using the likelihood ratio test and the backward selection procedure. Log transformations were conducted for RMSSD/SDNN and LF/HF rates. As the variables RMSSD and SDNN were highly correlated (r=0.90), only the results for RMSSD will be presented.

## 3.3 RESULTS

### 3.3.1 Behavioural data

A significant interaction between treatment and phase was noted for body posture changes. Sheep belonging to the HUM treatment showed lower number of body posture changes before the procedure than sheep belonging to the BRU treatment ($P<0.05$), and during the procedure, HUM sheep showed higher number of body posture changes than BRU sheep ($P<0.05$) (TABLE 5). In post-procedure phase, no significant difference was found in both treatments ($P>0.05$) (TABLE 5). For BRU sheep, body posture changes were more frequent before ($P<0.05$) and after ($P<0.05$) than during brushing (TABLE 5).

TABLE 5 - ESTIMATES AND STANDARD-ERRORS OF BEHAVIOURAL VARIABLES ASSESSED AS MEAN NUMBER OF CHANGES, SUCH AS BODY POSTURES, HEAD ORIENTATION AND EAR POSTURES, CONSIDERING THE EFFECTS OF TREATMENT, HUMAN PRESENCE (HUM) AND BRUSHING (BRU), PHASE (PRE-, DURING AND POST-PROCEDURES) AND GENETIC LINE, HIGHLY (R+) OR LOWLY (R-) REACTIVE TO SOCIAL SEPARATION

| Variables assessed as mean number of changes | Interaction Treatment\|Phase | | | | Interaction Treatment\|Genetic line | | Main effect Genetic line | | Main effect Phase | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Treatment | Phase | | | Treatment | Genetic line | | | | | |
| | | Pre | During | Post | | R+ | R- | R+ | R- | Pre | During | Post |
| Body posture changes | HUM | $0.50^{Aa*}$ (0.23) | $1.23^{Aa}$ (0.35) | $1.34^{Aa}$ (0.51) | HUM | ns | | ns | | | na | |
| | BRU | $2.06^{Ab}$ (0.78) | $0.25^{Bb}$ (0.17) | $2.06^{Aa}$ (0.70) | BRU | | | | | | | |
| Head orientation changes | HUM | $13.92^{Aa}$ (1.96) | $14.08^{Aa}$ (2.32) | $11.91^{Aa}$ (3.16) | HUM | $16.25^{Aa}$ (2.44) | $10.84^{Aa}$ (1.82) | na | | | na | |
| | BRU | $16.68^{Aa}$ (1.67) | $2.71^{Bb}$ (1.28) | $10.78^{Aa}$ (2.38) | BRU | $7.07^{Ab}$ (1.31) | $8.75^{Aa}$ (1.90) | | | | | |
| Ear changes | HUM | $8.81^{Aa}$ (1.32) | $10.77^{Aa}$ (1.73) | $7.59^{Aa}$ (1.28) | HUM | ns | | $10.83^{A}$ (1.06) | $7.68^{B}$ (0.87) | | na | |
| | BRU | $15.69^{Ab}$ (2.71) | $5.36^{Bb}$ (1.13) | $9.52^{Ba}$ (1.92) | BRU | | | | | | | |

*DIFFERENT CAPITAL LETTERS IN COLUMNS REFER TO SIGNIFICANT DIFFERENCES BETWEEN PHASES AND GENETIC LINES (P<0.05); DIFFERENT LOWER CASE LETTERS IN LINES INDICATE SIGNIFICANT DIFFERENCES BETWEEN TREATMENTS (P<0.05); NS = NON SIGNIFICANT; NA = NOT APPLICABLE.

Interactions between treatment and phase, and treatment and genetic line, were significant for the number of head orientation changes (TABLE 5). During the procedure, HUM sheep showed higher number of head orientation changes than BRU sheep (P<0.001) (TABLE 5). Only for BRU sheep, more frequent head orientation changes were observed before (P=0.001) and after than during brushing (P<0.05) (TABLE 5). Concerning the genetic lines, a higher number of head orientation changes was found for HUM rather than BRU sheep, only for R+ ewes (P=0.001) (TABLE 5).

Similarly, the interaction between treatment and phase and the main effect of genetic line were significant for the number of ear posture changes (TABLE 5). Higher number of ear changes was observed for the BRU group rather than the HUM group in pre-procedure phase (P=0.01) (TABLE 5). However, during the procedure, the opposite was noted: higher proportion of ear changes was found for the HUM group (P<0.01) (TABLE 5). For BRU sheep, ear changes were more frequent before than during brushing (P<0.001) (TABLE 5). Concerning the genetic lines, R+ ewes showed higher number of ear changes than R- ewes (P<0.05) (TABLE 5).

The main effects of phase and genetic line were significant for raised up ear postures. Significant differences were observed before and during the procedures, as sheep performed raised up ear postures for longer pre- rather than during the procedures ($P<0.05$) (TABLE 6). Concerning the genetic line, R- ewes showed raised up ears for longer when compared to R+ ewes ($P<0.001$) (TABLE 6). When the asymmetric posture was considered in the analyses, similar results were found, as the interaction between treatment and genetic line and the main effect of phase were significant. Among R+ sheep, animals submitted to the HUM treatment showed raised up or asymmetric ear postures for longer, when compared to animals in the BRU group ($P<0.05$) (TABLE 6). Among BRU sheep, R- ewes performed raised up or asymmetric ears for longer than R+ sheep ($P<0.001$) (TABLE 6). The asymmetric posture was expressed for longer before when compared to during the procedures ($P<0.05$) (TABLE 6). Higher proportion of time spent on the horizontal posture was observed during than before the procedures ($P<0.05$) (TABLE 6).

TABLE 6 - ESTIMATES AND STANDARD-ERRORS OF BEHAVIOURAL VARIABLES ASSESSED AS MEAN PROPORTION OF TIME, SUCH AS EAR POSTURES, EYE APERTURE, TAIL WAGGING AND FEEDING BEHAVIOUR, OBTAINED FROM MODEL ADJUSTMENTS, CONSIDERING THE EFFECTS OF TREATMENT, HUMAN PRESENCE (HUM) AND BRUSHING (BRU), PHASE (PRE-, DURING AND POST-PROCEDURES) AND GENETIC LINE, HIGHLY (R+) OR LOWLY (R-) REACTIVE TO SOCIAL SEPARATION

| Variables assessed as mean proportion of time | Interaction Treatment\|Phase | | | | Interaction Treatment\|Genetic line | | | Main effect Genetic line | | Main effect Phase | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Treatment | Phase Pre | During | Post | Treatment | Genetic line R+ | R- | R+ | R- | Pre | During | Post |
| Raised up ear posture | HUM | ns | | | HUM | ns | | $0.42^{A}$ (0.05) | $0.67^{B}$ (0.05) | $0.66^{A}$ (0.05) | $0.45^{B}$ (0.06) | $0.53^{AB}$ (0.07) |
| | BRU | | | | BRU | | | | | | | |
| Raised up + Asymmetric ear postures | HUM | ns | | | HUM | $0.63^{Aa}$ (0.06) | $0.65^{Aa}$ (0.07) | na | | $0.73^{A}$ (0.05) | $0.53^{B}$ (0.06) | $0.64^{AB}$ (0.06) |
| | BRU | | | | BRU | $0.45^{Ab}$ (0.05) | $0.79^{Ba}$ (0.05) | | | | | |
| Eye aperture | HUM | $0.08^{Aa}$ (0.04) | $0.15^{Aa}$ (0.04) | $0.22^{Aa}$ (0.07) | HUM | ns | | $0.25^{A}$ (0.04) | $0.13^{B}$ (0.03) | na | | |
| | BRU | $0.08^{Aa}$ (0.04) | $0.53^{Bb}$ (0.06) | $0.22^{Aa}$ (0.05) | BRU | | | | | | | |
| Tail wagging | HUM | $0.01^{Aa}$ (0.002) | $0.01^{Aa}$ (0.003) | $0.007^{Aa}$ (0.002) | HUM | ns | | ns | | na | | |
| | BRU | $0.02^{Aa}$ (0.005) | $0.16^{Bb}$ (0.05) | $0.02^{Ab}$ (0.009) | BRU | | | | | | | |
| Feeding behaviour | HUM | ns | | | HUM | $0.12^{Aa}$ (0.06) | $0.26^{Aa}$ (0.07) | na | | ns | | |
| | BRU | | | | BRU | $0.48^{Ab}$ (0.08) | $0.10^{Ba}$ (0.05) | | | | | |

*DIFFERENT CAPITAL LETTERS IN COLUMNS REFER TO SIGNIFICANT DIFFERENCES BETWEEN PHASES AND GENETIC LINES (P<0.05); DIFFERENT LOWER CASE LETTERS IN LINES INDICATE SIGNIFICANT DIFFERENCES BETWEEN TREATMENTS (P<0.05); NS = NON SIGNIFICANT; NA = NOT APPLICABLE.

A significant interaction between treatment and phase and the main effect of genetic line were observed for eye aperture. It was observed that BRU sheep showed closed and half-closed eyes for longer during brushing, when compared to the phases before (P<0.001) and after brushing (P<0.05) (TABLE 6). It was also noted that HUM sheep had a lower proportion of closed and half-closed eyes compared to BRU sheep, during the procedure (P<0.001) (TABLE 6). Highly reactive sheep performed closed and half-closed for longer than R- sheep (P=0.01) (TABLE 6).

The interaction between treatment and phase was significant for tail wagging. Brushed ewes wagged their tails for longer during the stimulus compared to the phases pre- (P<0.001) and after brushing (P=0.001) (TABLE 6). During (P<0.001) and after the procedure (P<0.05), BRU sheep wagged their tails for longer than HUM sheep (TABLE 6).

The interaction between treatment and genetic line was significant for feeding behaviour. Among R+ ewes, BRU sheep spent more time eating and ruminating when compared to HUM sheep ($P<0.01$) (TABLE 6). Among the animals belonging to the BRU group, R+ sheep showed a higher proportion of time eating and ruminating, in comparison with R- sheep ($P<0.001$) (TABLE 6).

### 3.3.2 Cardiac data

A significant effect of interaction between treatment and genetic line and the main effect of phase were noted for HR. Among HUM sheep, R+ ewes showed, on average, 8.77±2.80 less bpm than R- sheep ($P<0.01$) (FIGURE 4). On the contrary, among BRU sheep, R+ sheep showed 9.06±2.66 more bpm, on average, than R- animals ($P<0.001$) (FIGURE 4). Among R- sheep, BRU animals presented lower HR than HUM animals, on average, 10.22±2.58 less bpm ($P<0.001$). Among R+ sheep, the opposite: sheep in the BRU group showed higher HR when compared to sheep in the HUM group, 7.61±2.87 more bpm, on average ($P<0.01$). Both HUM and BRU sheep had lower HR during and post-procedure, when compared to the phase pre-procedure (FIGURE 4); during and after the procedures, it was estimated that sheep had, on average, 2.17±0.49 less bpm ($P<0.001$) and 1.97±0.49 less bpm ($P<0.001$), respectively, than in pre-procedures phases.

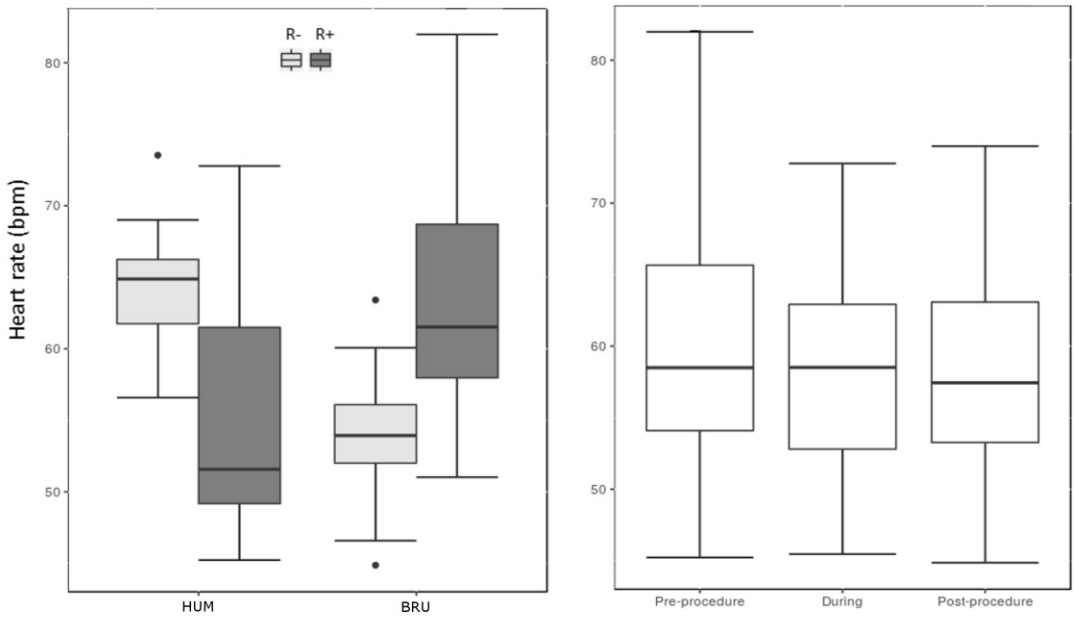


FIGURE 4 - HEART RATE (BPM) RECORDED IN 17 HIGHLY (R+) AND 21 LOWLY (R-) REACTIVE EWES SUBMITTED TO HUMAN PRESENCE (HUM) AND BRUSHING (BRU), ASSESSED IN PRE-, DURING AND POST-PROCEDURE PHASES

No significant differences were found for RMSSD ($P>0.05$) (FIGURE 5). However, the main effects of phase and genetic line were significant for RMSSD/SDNN. RMSSD/SDNN rates were higher mainly during HUM and BRU, when compared to pre- and post-procedure phases (FIGURE 6). An average decrease of 0.07±0.03 ($P<0.05$) and 0.08±0.03 ($P<0.01$) on (log) RMSSD/SDNN was estimated before and after, in comparison with the phase during the procedures, respectively. Higher RMSSD/SDNN was also found for R- sheep rather than R+ sheep ($P=0.001$) (FIGURE 6), i.e. an estimated average increase of 0.14±0.05 on (log) RMSSD/SDNN for R- sheep.

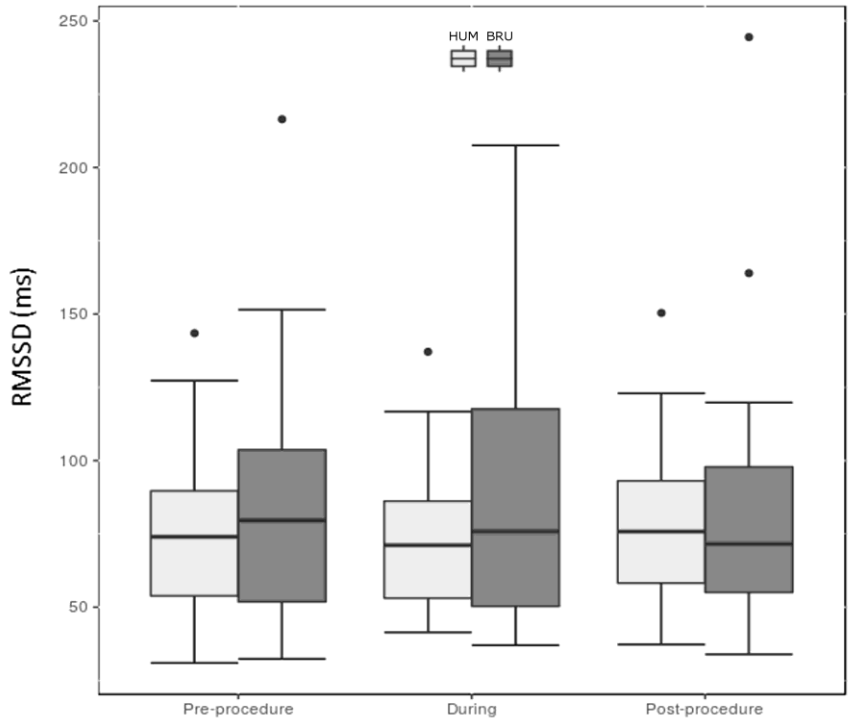


FIGURE 5 - RMSSD RECORDED IN 17 HIGHLY (R+) AND 21 LOWLY (R-) REACTIVE EWES SUBMITTED TO HUMAN PRESENCE (HUM) AND BRUSHING (BRU), ASSESSED IN PRE-, DURING AND POST-PROCEDURE PHASES

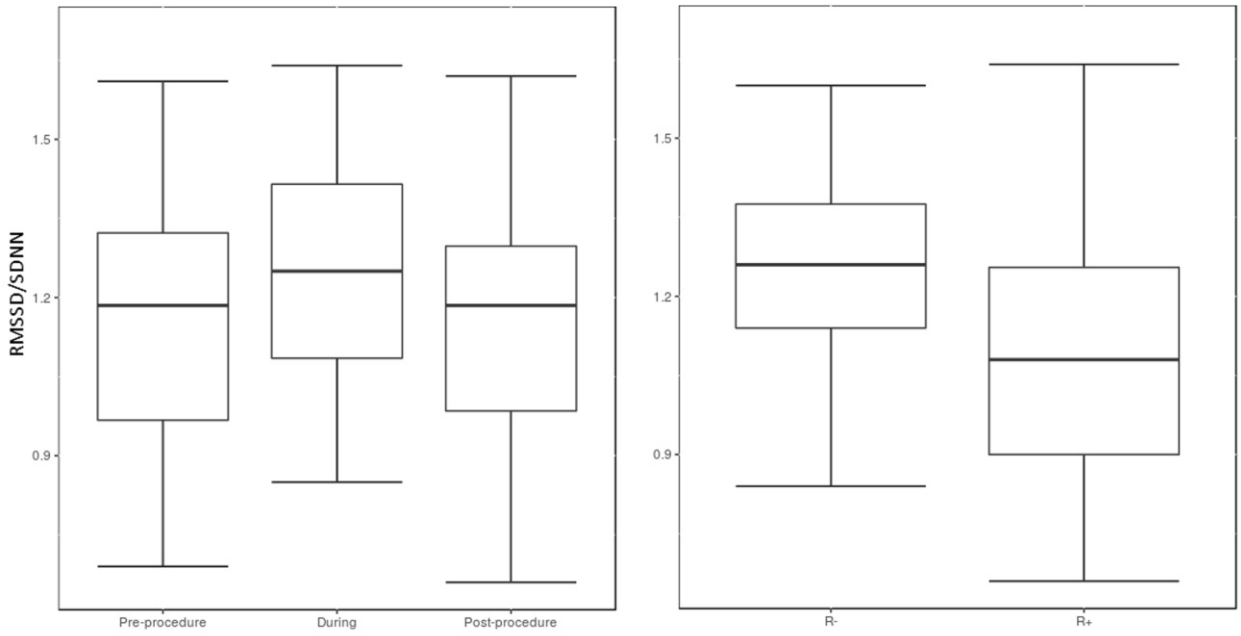


FIGURE 6 - RMSSD/SDNN RECORDED IN 17 HIGHLY (R+) AND 21 LOWLY (R-) REACTIVE EWES SUBMITTED TO HUMAN PRESENCE (HUM) AND BRUSHING (BRU), ASSESSED IN PRE-, DURING AND POST-PROCEDURE PHASES

Results in the frequency domain data showed the same trend as the responses observed for time domain variables, as interactions between treatment

and phase, as well as treatment and genetic line, were significant for LF/HF. After the procedure, BRU sheep showed higher LF/HF than HUM sheep (P<0.05) (FIGURE 7), with an average increase of 0.78±0.32 on (log) LF/HF. Among BRU sheep, an increase in LF/HF was found in post-brushing phase as compared to pre- and during brushing (P<0.05) (FIGURE 7), with an average increase of 0.57±0.24 on (log) LF/HF in post-brushing phase. Among HUM sheep, R+ animals showed higher LF/HF than R- animals (P<0.01) (FIGURE 7), with an estimated average increase of 0.92±0.31 on (log) LF/HF.

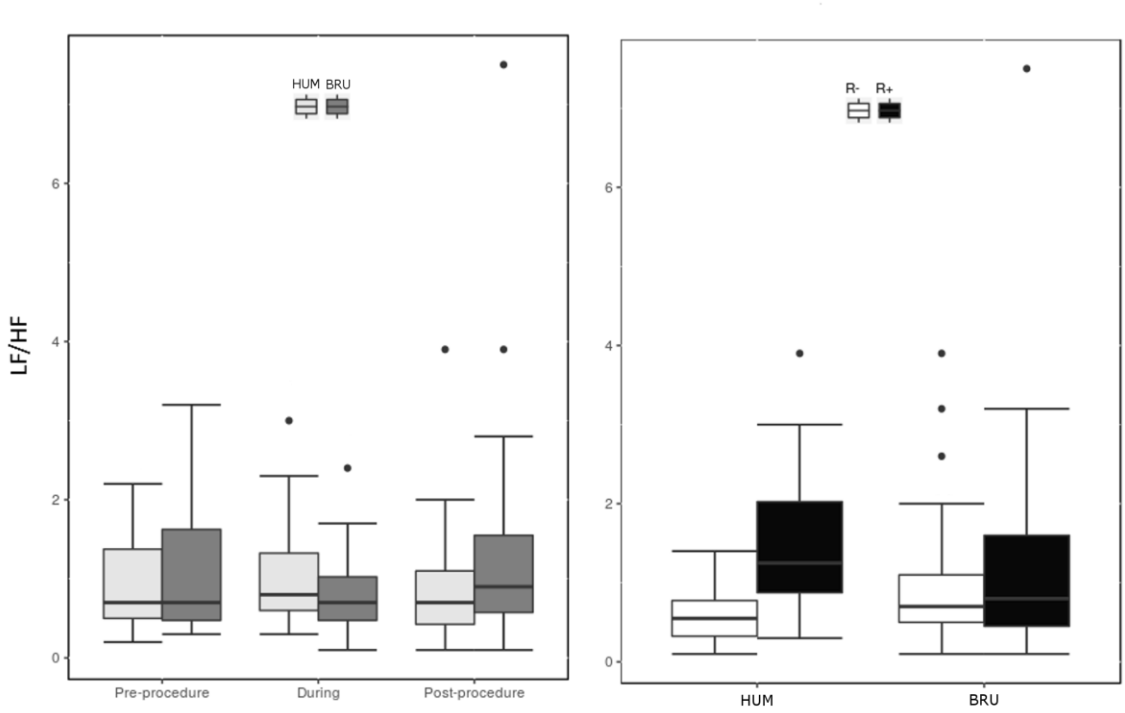


FIGURE 7 - LF/HF RECORDED IN 17 HIGHLY (R+) AND 21 LOWLY (R-) REACTIVE EWES SUBMITTED TO HUMAN PRESENCE (HUM) AND BRUSHING (BRU), ASSESSED IN PRE-, DURING AND POST-PROCEDURE PHASES

## 3.4 DISCUSSION

All the behavioural indicators confirmed the hypothesis that sheep experienced a more positive state during BRU as compared to HUM. Vigilance and higher attention seemed to be expressed differently through ear postures and changes by R- and R+ ewes, demanding further research. Data on HR and RMSSD/SDNN showed that the animals expressed relaxing responses towards both procedures. Furthermore, anticipatory responses to brushing suggest that sheep showed a positive expectation to be brushed. Results on head orientation changes,

feeding behaviour and LF/HF ratio suggest that brushing might have a more calming effect over R+ sheep.

Higher number of body posture, head orientation and ear posture changes before brushing suggest anticipation of a positive reward, which was also observed in silver foxes (MOE et al., 2006). An increase in body moves after brushing seems to point to higher expectation of BRU sheep to be brushed for longer. Lower proportion of body moves, head orientation and ear posture changes, in addition to higher duration of closed and half-closed eyes, tail wagging and feeding behaviour during brushing suggest that sheep experienced a positive state when brushed. Most results are in accordance with literature findings for ruminants (ear posture changes: REEFMANN et al., 2009; REEFMANN; WECHSLER; GYGAX, 2009; BOISSY et al., 2011; REEFMANN et al., 2012; ear postures: REEFMANN et al., 2009; REEFMANN; WECHSLER; GYGAX, 2009; REEFMANN et al., 2012; COULON et al., 2015; eye aperture: SANDEM; BRAASTAD; BOE, 2002; SANDEM; JANCZAK; BRAASTAD, 2004; SANDEM; BRAASTAD; BAKKEN, 2006; REEFMANN; WECHSLER; GYGAX, 2009; PROCTOR; CARDER, 2015; feeding behaviour: VÖGELI et al., 2015); there is a lack of literature data on the relation between tail wagging and emotional states in sheep (GRANT, 2004; REEFMANN et al., 2009; TAMIOSO et al., 2017). It is quite rare to observe tail wagging in sheep, especially due to tail docking; lambs raise and wag their tails while suckling, for example. Assuming that suckling and brushing are positive stimuli for sheep, our results suggest that tail wagging may be a useful indicator of positive affective states in sheep. It seems unlikely that the behavioural responses noted during brushing may be explained by reasons other than a positive affective state, since it was permanently possible for the ewes to move away from human contact, and it is reported that animals seek stimuli that elicit positive emotions and avoid aversive ones (MELLOR, 2012). The behavioural indicators pointed to a more positive perception towards the brushing treatment.

Highly reactive sheep submitted to the HUM treatment showed higher number of head orientation changes than BRU, suggesting that brushing had a more calming effect on highly reactive animals. Beausoleil et al. (2008) also found that more active sheep, i.e. animals that showed high levels of locomotor activity in the arena test and high levels of agitation in the box test, were less fearful than less active sheep when tested toward an unfamiliar observer. Highly reactive sheep also showed higher number of ear posture changes in response to both procedures as

compared to R- sheep, which may be related to higher vigilance conditions, regardless of the stimulus. The results for raised up and asymmetric ear postures, concerning vigilant states, present a different behavioural pattern between R+ and R- sheep: R- ewes in the BRU group performed raised up or asymmetric ears for longer than R+ ewes, which suggests higher attention from R- sheep. The findings indicate that higher attention might have been expressed differently by R+ and R- ewes: R+ sheep showed more ear posture changes, whereas R- performed more raised up and asymmetric ear postures. In summary, sheep belonging to different genetic lines selected to high and low reactivity to social separation showed different ways of expression of ear changes and postures. In a previous study, we also observed unexpected differences in ear postures due to a small breed variation between Dorper and White Dorper sheep (TAMIOSO et al., 2017). Therefore, the effect of breed differences on ear posture responses warrants further studies. Findings for closed and half-closed eyes and feeding behaviour allow for a similar conclusion, that brushing might have elicited a more calming state on R+ sheep.

In general, cardiac data showed that both stimuli elicited relaxing responses in sheep, mainly during the procedures. Data on HR suggested that human presence elicited lower arousal state in R+ and higher arousal state in R- sheep; for brushing, the opposite was found. Differences in the way R+ and R- sheep responded to the stimuli were expected, as selection for reactivity to social isolation is likely to be accompanied by cardiac reactivity changes; the study of these differences may provide important insight on how reactivity may influence sheep autonomic responses. As, to our knowledge, this is the first study to explore the effect of human presence and brushing in sheep belonging to different genetic lines regarding reactivity to social isolation, it is difficult to compare results with literature data. More studies on the association between behavioural and cardiac indicators with positive stimuli in animals selected for reactivity are encouraged. In a research on two divergent lines of quails, pharmacological blockades demonstrated that selection on tonic immobility duration was associated with changes in the nervous control of heart rate and that the dominant subsystem differed according to the quail line (VALANCE et al., 2007). Our findings also indicate that autonomic responses are influenced by genetic background.

General low HR during HUM and BRU confirm some behavioural results, as those for ear postures, on the relaxing state the sheep experienced during each

stimulus, and its carry-over effect immediately after the stimulus ended. Additionally, anticipation of presumed positive events has been found to be related to sympathetic activation and reflect increased arousal (BRAESICKE et al., 2005; MAHNHARDT et al., 2014; LELIVELD et al., 2016). We noted that, in general, sheep from both HUM and BRU groups presented low HR, equal to 57.9±7.3 bpm and 60.1±7.7 bpm, respectively. However, it was expected that BRU sheep would show lower HR, as gentle tactile interactions are supposed to trigger a calming state in farm animals, with a reduction in heart rate (SCHMIED; BOIVIN; WAIBLINGER, 2008). Human presence per se may have elicited a relaxing state in the ewes as brushing was expected to; some studies show that the presence of a familiar experimenter may calm the animals in negative situations (WAIBLINGER et al., 2004; LELIVELD et al., 2016). As previous experience including those with stock people influence animals' perception of humans (HEMSWORTH; BOIVIN, 2011), the findings on HR suggested that both human presence and brushing were judged as positive by the animals.

No effect was significant for RMSSD. Considering that RMSSD reflects the activity of parasympathetic branch, and that presumed positive situations are usually characterized by higher values of RMSSD (COULON et al., 2015), we expected higher RMSSD values for BRU ewes. However, the absence of differences in RMSSD was also reported in other studies on sheep, pigs and dogs (REEFMANN et al., 2012; MAHNHARDT et al., 2014; TRAVAIN et al., 2016). According to HR and RMSSD data, both human presence and brushing seem to be similar in valence and arousal, eliciting relatively lower intensity and similar responsiveness of the parasympathetic system. In addition, both treatments might have been perceived similarly by R+ and R- sheep, according to RMSSD responses. As reported by Gygax et al. (2013), RMSSD may not be a suitable indicator of emotional arousal, and probably of valence; the authors found no significant differences in RMSSD ratios in dwarf goats submitted to situations differing in valence (feed reward and frustration). Different results on the use of RMSSD in studies of emotions in animals suggest the necessity of further studies aiming to record RMSSD in different situations and probably for longer durations. Even though no significant difference was found in our study, a growing body of literature shows that RMSSD is a valuable indicator of the parasympathetic nervous tone and may be used to assess positive emotions in animals. The same may be applied to SDNN, as it was highly correlated with RMSSD, in our study, and it is also an important measure of affective states in

animals, reflecting both vagal and sympathetic influences of HR (VON BORELL et al., 2007). Our results indicate that important differences may be better exposed when RMSSD is reported in relation to SDNN.

As found for HR, an increase in RMSSD/SDNN ratio mainly during the procedures indicates an increase of the vagal tone, or a decrease in the activation of the sympathetic system, which may be associated with positive affective states. In a study on learning behaviour and autonomic reactions in pigs, Zebunke et al. (2011) observed that, during feeding, the RMSSD/SDNN ratio was elevated, indicating an increase in the parasympathetic activity. Higher RMSSD/SDNN observed in R- group suggests a stronger activation of the parasympathetic system in R- ewes in response to both familiar human and brushing. Such results are in accordance with previous studies that demonstrated the activation of the sympathetic system in more reactive, temperamental animals, i.e. cows that were behaviourally selected by head shaking, defense reactions or fall during a restriction test in which they were tethered (KOVÁCS et al., 2015). Increased sympathetic activity shown by R+ sheep in our study may be linked to increased arousal in response to both human presence and brushing. The findings support that RMSSD/SDNN ratio is an important indicator of sympathovagal influences on heart rate. The frequency domain measures of heart rate seem worth of further investigation since many of our comparisons demonstrated statistical significance, suggesting the sensibility of the indicators, and our current understanding of their meaning, as judged by available literature, appears limited.

Higher LF/HF ratio was noted mainly after brushing. A possible explanation for significant alterations in LF/HF after brushing may be that BRU sheep expected to be brushed for longer, as observed for body postures. As an indirect correspondence of RMSSD, the HF power is an index of parasympathetic activity, whereas LF power is an indicator of both sympathetic and parasympathetic activities. Thus, higher values of HF would indicate an activation of the parasympathetic system, which may reflect positive emotional states (MCCRATY et al., 1995). Yet there are few studies on LF/HF ratio and its relationship with emotional states in animals. Pig victims of tail-biting, an obvious negative situation, showed higher LF/HF ratio during basal conditions, probably related to a more suppressed parasympathetic tone (ZUPAN et al., 2012). In cattle submitted to stressful events, Mohr, Langbein and Nurnberg (2002) observed significant decreases in HF rate following external stress and even

marked decline in response to pathological load. The authors found no significant difference for LF. The LF/HF ratio significantly increased from the group of animals submitted to no stress load to the group submitted to internal stress load (diarrhea). The results showing that R+ ewes from the HUM group had higher LF/HF than R- ewes may be related to an activation of the sympathetic tone of R+ sheep toward human presence. Temperamental cows also showed higher LF/HF when compared with calm and intermediate animals assessed in lying posture (KOVÁCS et al., 2015). In smaller-scale farms, Kovács et al. (2015) observed that intermediate cows showed lower LF/HF, i.e. higher vagal and lower sympathetic activity than temperamental cows. The results from the literature indicate that higher LF/HF ratio seem to be a good indicator of the perception toward negative events, whereas the opposite, i.e., lower LF/HF would reflect vagal modulation. In our study, higher LF/HF after brushing might indicate higher expectancy to be brushed by BRU ewes and higher LF/HF in R+ sheep, the activation of the sympathetic tone in response to human presence.

## 3.5 CONCLUSION

Behavioural indicators suggested that sheep experienced a more pleasurable state during brushing than when submitted to the mere human presence. Brushed sheep performed fewer body postures, head orientation and ear posture changes and longer duration of closed and half-closed eyes, tail wagging and rumination when compared to non-brushed animals. Brushed sheep also showed more body posture, head orientation and ear posture changes before the brushing procedure, suggesting anticipation of a positive event. The cardiac indicators showed that either human-accompanied sheep or brushed sheep experienced a calming state. Heart rate and RMSSD/SDNN ratio reflected the activation of the parasympathetic nervous system in both treatments, which is generally associated with a relaxing state. Genetic effects were also significant over some variables. The fact that R+ sheep expressed less head orientation changes, longer duration of feeding and ruminating in response to brushing and higher LF/HF in response to human presence indicates that gentle physical contact with a familiar human elicits a more relaxing effect in R+ animals.

## 3.6 ACKNOWLEDGMENTS

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# 4. DOES EMOTIONAL REACTIVITY ALTER THE RELAXING RESPONSES OF BRUSHED EWES?

## RESUMO

O estímulo tátil gentil elicia estados emocionais positivos em ovinos. Objetivou-se investigar se a reatividade emocional e o contexto social influenciam as respostas comportamentais e cardíacas de ovelhas submetidas ao contato tátil positivo. Vinte ovelhas da raça Romane, selecionadas para baixa (R-) ou alta (R+) reatividade comportamental ao isolamento social, foram regularmente escovadas por um avaliador familiar. O experimento foi conduzido em três sessões, sendo que nas sessões 1 e 3 foi utilizada uma barreira separando o animal escovado e membros do grupo, sem distância entre eles, e na sessão 2, foram utilizadas duas barreiras separando o animal escovado e membros do grupo por uma distância de 1,7 m. Posturas corporais, orientação da cabeça, mudanças e posturas de orelha, grau de abertura do olho, movimentação da cauda e comportamento alimentar, além da frequência cardíaca (FC) e da variabilidade da frequência cardíaca (RMSSD, SDNN, RMSSD/SDNN e LF/HF) foram avaliados. As variáveis foram analisadas utilizando modelos lineares generalizados e modelos lineares mistos. Sessão, linhagem genética e fase (pré-, escovação e pós-escovação) foram consideradas efeitos fixos, incluindo suas interações. De maneira geral, a separação social na sessão 2 não alterou as respostas das ovelhas, uma vez que não houve diferença na sessão 2 ($P>0,05$); foram observadas menos mudanças nas posturas de orelha na sessão 3 do que na sessão 1 ($P<0,01$) e a razão RMSSD/SDNN foi maior principalmente durante a escovação nas sessões 1 e 3 ($P<0,05$). Entretanto, a separação social influenciou as respostas de ovelhas R+ e R-, por meio de dados de posturas de orelha e FC; ovelhas R+ expressaram posturas de orelha assimétricas por mais tempo na sessão 2 do que nas sessões 1 e 3 ($P<0,01$), e na sessão 3 do que na sessão 1 ($P<0,01$) indicando que a separação dos membros do grupo teve efeito negativo sobre ovelhas R+. Ovelhas menos reativas expressaram posturas de orelha horizontais por menos tempo na sessão 2 do que nas sessões 1 e 3 ($P<0,001$) e ovelhas R+ apresentaram posturas de orelha horizontais por mais tempo na sessão 1 do que na sessão 3 ($P<0,001$). Encontrou-se também maior FC entre ovelhas R- na sessão 2 do que nas sessões 1 e 3 ($P<0,001$) e na sessão 3 do que na sessão 1 ($P=0,001$). Ovelhas R+ apresentaram maior FC na sessão 1 do que na sessão 3 ($P<0,001$). Os resultados sugerem que o contexto social pode influenciar a reatividade emocional das ovelhas ao manejo gentil.

Palavras-chave: Comportamento. Manejo positivo. Ovinos. Reatividade emocional. Sistema nervoso autônomo.

## ABSTRACT

Gentle tactile stimulation elicits positive emotional states in sheep. We investigated whether emotional reactivity and social context influence behavioural and cardiac responses of sheep to gentle stimulation. Twenty Romane ewes, selected for low (R-) or high (R+) behavioural reactivity to social isolation, were regularly brushed by a familiar human. The experiment was conducted in three sessions, being sessions 1 and 3 with one grid separating the brushed animal from pen mates, with no distance between them, and session 2 with two grids, separating the brushed animal from pen mates by a distance of 1.7 m. Body postures, head orientation, ear changes and postures, eye aperture, tail wagging and feeding behaviour, in addition to heart rate (HR) and heart rate variability (RMSSD, SDNN, RMSSD/SDNN and LF/HF ratios) were assessed. The variables were analyzed using generalized linear models and linear mixed models. Session, genetic line and phase (pre-, brushing and post-brushing) were considered fixed effects, including their interactions. Overall, social separation did not alter sheep responses as no difference was observed in session 2 ($P>0.05$); fewer ear posture changes were noted in session 3 than in session 1 ($P<0.01$) and the RMSSD/SDNN ratio was higher mainly during brushing in sessions 1 and 3 ($P<0.05$). However, social separation influenced R+ and R- sheep responses, according to ear posture and HR data; R+ sheep performed asymmetric ear postures for longer in session 2 than in sessions 1 and 3 ($P<0.01$), and in session 3 than in session 1 ($P<0.01$), indicating that the separation of pen mates had a negative effect over R+ sheep. Lowly reactive sheep spent less time on horizontal ear postures in session 2 than in sessions 1 and 3 ($P<0.001$), and R+ sheep spent more time on horizontal postures in session 1 than in session 3 ($P<0.001$). It was also found higher HR among R- sheep in session 2 than in sessions 1 and 3 ($P<0.001$), and in session 3 than in session 1 ($P=0.001$). Highly reactive sheep showed higher HR in session 1 than in session 3 ($P<0.001$). The findings suggest that the social context might influence emotional reactivity of sheep to gentle handling.

Keywords: Behaviour. Positive handling. Sheep. Emotional reactivity. Autonomic Nervous System.

## 4.1 INTRODUCTION

Reactivity is defined as the behavioural and physiological responses to environmental changes and challenges (MANTECA; DEAG, 1993). Emotional reactivity refers to the predisposition of an individual to being frightened (JONES, 1996). A large variability in emotional reactivity, also known as fearfulness, has been demonstrated in animals (LANIER et al., 2000). Emotional reactivity also has an important influence on the way animals interact with and respond to the environment (BOISSY, 1995). Evidences suggest a relationship between emotional reactivity and different rearing conditions in animals (ROY et al., 2001; KOSEKI et al., 2012).

Research suggest that positive stimuli as environmental enrichment for cows may improve their quality of life (BERTENSHAW; ROWLINSON, 2008); in this sense, physical interactions, as stroking and brushing, seem to be associated with positive emotional states in animals. Tactile contact has been related to the expression of relaxing behavioural and cardiac responses in sheep and cattle, as fewer ear posture changes, more axial ear postures, tail wagging and presence of half-closed eyes, and lower heart rate (HR) and increased square root of the mean squared differences of successive NN intervals (RMSSD) (SCHMIED; BOIVIN; WAIBLINGER, 2008; REEFMANN; WECHSLER; GYGAX, 2009; COULON et al., 2015; TAMIOSO et al., 2017).

However, no research has assessed the influence of emotional reactivity on the responses of farm animals toward gentle tactile stimulation. We aimed to study whether social separation influences general behavioural and cardiac responses of sheep, using physical barriers separating the brushed animal from group members, and whether social separation effects are influenced by emotional reactivity, studying lowly and highly reactive sheep to temporary social separation. We hypothesize that brushing while close to group members triggers the expression of more relaxing behavioural responses, with fewer body posture, head orientation and ear posture changes, longer duration of horizontal ear postures, closed and half-closed eyes, tail wagging and feeding behaviour, in addition to lower HR, standard deviation of all inter-beat intervals (SDNN), ratio between low-frequency (LF) and higher frequency (HF) powers (LF/HF rate) and higher RMSSD and RMSSD/SDNN, mainly in lowly reactive sheep. On the opposite, when distant from group members, highly reactive animals present more body posture, head orientation and ear posture changes, and

lower duration of horizontal ear postures, closed and half-closed eyes, tail wagging and feeding behaviour, as well as higher activation of the sympathetic system.

## 4.2 MATERIAL AND METHODS

### 4.2.1 Animals

The experiment was performed at INRA experimental farm La Fage, Roquefort, France in September, 2015. Twenty 15-month-old non-gestating and non-lactating Romane sheep were assessed. They belonged to two genetic lines, selected according to their behavioural reactivity towards temporary social separation: low (R-) and high (R+) reactivity when separated from their group. Before the experiment, the ewes were kept outdoors as a single flock. The animals were born on the farm where the research was conducted and they were submitted to their regular management conditions during the experiment. Water was available ad libitum and the ewes were fed with hay twice a day, before (06:00 am) and after (18:00 pm) testing sessions. Six days before the beginning of testing sessions, they were taken from the pasture and moved to indoor pens of approximately 16 m², fitted with straw bedding and a feeding area, inside a shed. They were randomly allocated in four groups of four to six animals. The sheep had partial to no visual contact with the animals from other pens. However, they could hear and smell the sheep from other pens. The approval of this experiment by an ethics committee was not obligatory in France, since the ewes were not submitted to any physical nociceptive stimulation. After the study, the ewes remained on the farm of origin, under the described rearing conditions.

### 4.2.2 Adaptation

Sheep were handled twice a day, over six consecutive days per week, during four weeks of the adaptation period. The animals were adapted towards the equipment to measure both behaviour and heart rate (two cameras, camcorder and heart rate monitor), one barrier to separate the brushed animal from group members and a stopwatch. Sheep were also adapted to the presence of two experimenters: a female experimenter (experimenter A) responsible for brushing and a male

experimenter (experimenter B) for bedding and food delivery, as well as for fitting the animals with an elastic belt, during assessments in the morning and afternoon, that was replaced by a heart rate monitor in testing sessions. During the first week of adaptation, the animals were assessed in groups and adapted to be in the presence of experimenter A. During the second week, the animals were adapted to the presence of the 15 x 7 cm bristle, plastic-handled brush and to the brushing. Ewes were brushed respecting a fixed group order, randomly determined within the group. Brushing speed ranged from 20 to 40 brushing strokes/min and touched the sheep skin of the ventral neck, lateral chest, withers and belly. During the third and fourth weeks, ewes were separated from group members in the experimental pen. For that, we used one metal grid of 1.80 m length and 1.24 m height, composed of five bars with 10-cm spaces between bars. Such grid allowed visual and olfactory interactions between sheep.

Two groups of animals were assessed in the morning, from 08:00 to 10:00, and two in the afternoon, from 13:30 to 15:30. The groups were evaluated following a fixed order of group and period of the day. Assessments lasted 6 min per animal, comprising three phases: Phase 1 - Pre-brushing: experimenter A stayed beside the grid separating the brushed animal and group members, approximately 40 cm far from the brushed animal, out of the experimental area, during 2 min; Phase 2: each animal was brushed in the experimental area for 2 min, and Phase 3 - Post-brushing: similar procedures performed during the first phase, for 2 min.

### 4.2.3 Testing sessions

Testing sessions occurred two days after the adaptation period was finished, during four consecutive days. Each animal was assessed respecting the procedures of the adaptation period, with minor differences in the phases; each testing session totaled 8 min, divided in: Phase 1 - Pre-brushing: 2 min 30 s; Phase 2 - Brushing: 3 min; and Phase 3 - Post-brushing: 2 min 30 s. During testing sessions, experimenter B fitted the animals a heart rate monitor and recorded cardiac information. The ECG was monitored by an adjustable chest belt with three integrated electrodes and a telemetric wireless transmitter (EMKA Technologies, Paris, France, EMKaPack 4G). Experimenter B took 3 min to put the monitor in each animal, including the application of a transmission gel, fitting of the equipment and verifying that it was

working correctly. All the animals were previously shaved in the area where the heart rate monitor and the electrodes were placed.

Testing was organized in three sessions: During Session 1 (days 1 and 2), the brushed animal was assessed as described for the adaptation period, only one metal grid was used to separate the brushed animal from group members. In Session 2 (day 3), we used two identical metal grids 1.70 m apart to separate the brushed animal from group members, so that the brushed animal was approximately 2 m further from them, and such procedure was a novelty to all the animals, i.e. they were not adapted to it. In Session 3 (day 4), the animals were reassessed, with the same setting as in Session 1.

### 4.2.4 Behavioural and cardiac responses

Body postures, head orientation, ear changes and postures, tail wagging and feeding behaviour were recorded using two cameras; eye aperture (closed and half-closed eyes) was registered using a camcorder. All the behavioural indicators were coded using The Observer XT version 11.5 (Noldus Information Technology, Wageningen, The Netherlands). The cardiac indicators HR (mean heart rate value), RMSSD (root mean square of successive differences, i.e. square root of the mean of the sum of the squares of differences between successive NN inter-beat intervals differences), SDNN (standard deviation of all NN intervals), RMSSD/SDNN ratio and the ratio between low-frequency power (LF) and high-frequency power (HF), LF/HF, were coded with EMKA ECGauto version 3.3.0.30 (EMKA Technologies, Paris, France).

### 4.2.5 Statistical analysis

The statistical analyses were performed in R version 3.2.2 (R DEVELOPMENT CORE TEAM, 2015). The packages lme4 and geepack were used for fitting the models, and the multcomp and lsmeans packages were used for the estimation of contrasts and tests. Generalized linear models were fitted for the behavioural variables, with parameters estimated by generalized estimation equations, in order to deal with correlations between outcomes. The intra-animal covariance structure was estimated using a sandwich estimator, preventing potential

problems arising from incorrect specifications. Linear mixed models were fitted by the maximum likelihood method for the cardiac variables, including random animal effects to incorporate the correlations. Session (1, 2 and 3), genetic line (R- and R+) and phase (pre-, brushing and post-brushing) effects and their interactions were considered. Concerning the sessions, we defined contrasts to compare the results of session 2 in relation to the average of sessions 1 and 3, as well as the comparison of results between sessions 1 and 3. We compared sessions 1 and 3 versus 2, and session 1 versus 3, to verify if there was any possible residual effect of session 2 over session 3. We performed Wald (for behavioural variables) and F tests (for cardiac variables) to determine the effect of the factors in the analysis. In both cases, adjusted p-values were reported when multiple contrasts were tested simultaneously. The significance level was set at $P<0.05$.

Body postures, head orientation and ear postures were analyzed considering the number of variations, and for ear postures, eye aperture, tail wagging and feeding behaviour, the proportion of time was considered. The logarithmic link function was considered for the variables calculated as function of number of variations, and the logit link function, for the variables calculated as proportion of time. For eye aperture, closed and half-closed eyes were analyzed together, due to the small number of animals with closed eyes. For ear postures, the analyses were performed from the most frequent positions observed during coding: raised up, asymmetric and horizontal postures. For feeding behaviour, the proportion of time spent eating and ruminating was analyzed together. For the variable LF/HF we applied the logarithmic transformation due to its level of asymmetry.

Results were presented as ratio of means (for variables analyzed as number of variations and LF/HF ratio), odds ratios (for variables analyzed considering the proportion of time) and mean differences (for HR and RMSSD/SDNN), as well as 95% confidence intervals (CI). Ratios of means and odds ratios were calculated from the first to the second group under comparisons. Thus, for example, a ratio of means equal to 2.5 for a hypothetical comparison "pre- vs brushing" indicates that, on average, sheep changed postures 2.5 times more pre- than during brushing, controlling for the effects of other factors. Similar interpretations can be drawn for variables related to the proportion of time, but relative to their respective odds ratios.

## 4.3 RESULTS

### 4.3.1 Behavioural responses

Figure 8 shows that ewes performed few changes in body postures, head orientation and ear postures, during brushing, especially in sessions 1 and 3, with medians close to 0.

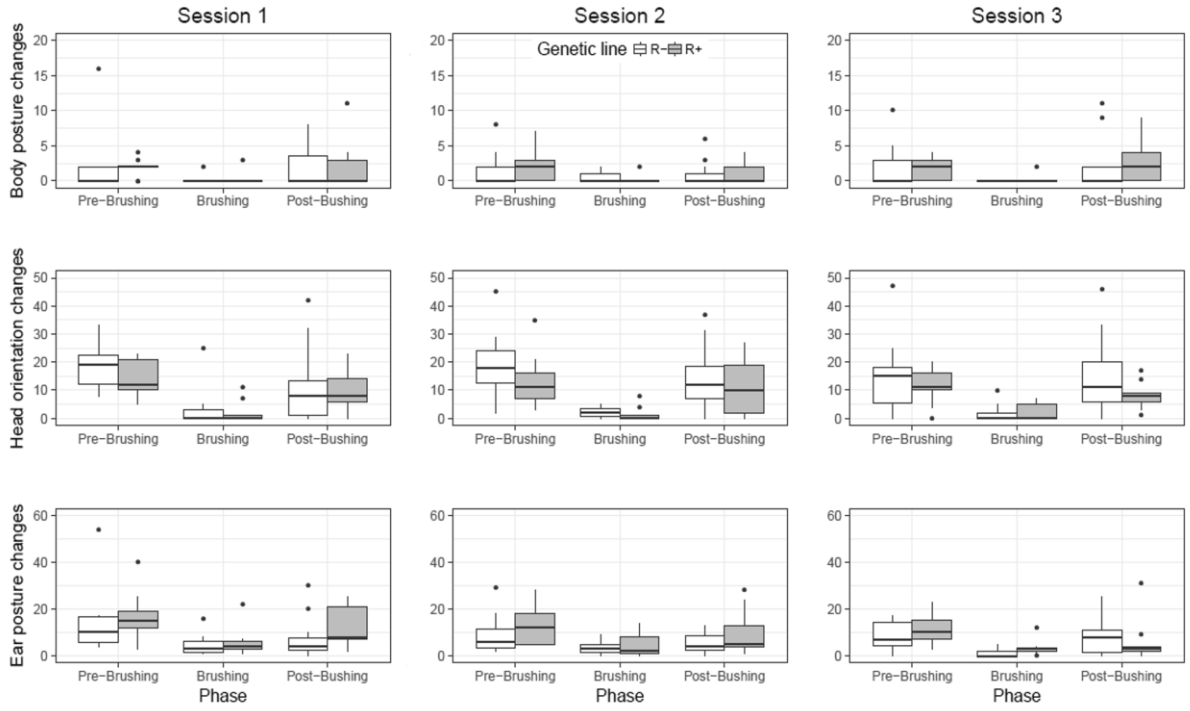


FIGURE 8 - BODY POSTURE, HEAD ORIENTATION AND EAR POSTURE CHANGES RECORDED IN 11 LOWLY (R-) AND 9 HIGHLY (R+) REACTIVE EWES, ASSESSED IN PRE-, DURING AND POST-BRUSHING PHASES, IN SESSIONS 1, 2 AND 3

From the adjustment of the models, it was noted that social separation did not alter sheep behavioural responses, as the main effect of session was only significant for ear posture changes ($P<0.05$) (TABLE 7). Fewer ear changes were noted during session 3 rather than in session 1 ($P<0.01$) (TABLE 7). No significant difference was found between sessions 1 and 3 in relation to session 2 for ear posture changes ($P>0.05$) (TABLE 7). Significant main effects of phase ($P<0.001$) and genetic line ($P<0.05$) were also observed for ear posture changes. Ear changes were more frequent pre- than during ($P<0.001$) and post-brushing ($P<0.05$); and post- than during brushing ($P<0.001$) (TABLE 7). Fewer ear posture changes were

observed for R- sheep than R+ sheep (P<0.05) (TABLE 7). For body postures and head orientation changes, only phase had a significant effect (P<0.001). Sheep showed more body posture and head orientation changes in pre- (P<0.001) and post-brushing (P<0.001) phases than during brushing (TABLE 7). We also found that the main effect of genetic line was significant for head orientation changes (P<0.05), as R- sheep showed more head orientation changes than R+ sheep (P<0.05) (TABLE 7).

TABLE 7 - RATIO OF MEANS (ROM) FOR BODY POSTURE, HEAD ORIENTATION AND EAR POSTURE CHANGES, IN 20 ROMANE EWES, CONSIDERING THE SIGNIFICANT EFFECTS OF PHASE (PRE-, BRUSHING AND POST-BRUSHING), SESSION (1, 2 AND 3) AND GENETIC LINE (R- AND R+)

| Variable/Effect | Comparison | RoM | CI (95%) | P |
|---|---|---|---|---|
| Body posture changes | | | | |
| Phase | Pre- vs brushing | 9.44 | 4.27; 20.86 | <0.001 |
| | Pre- vs post-brushing | 1.09 | 0.63; 1.88 | 0.94 |
| | Brushing vs post-brushing | 0.11 | 0.05; 0.26 | <0.001 |
| Head orientation changes | | | | |
| Phase | Pre- vs brushing | 7.24 | 4.41; 11.89 | <0.001 |
| | Pre- vs post-brushing | 1.30 | 0.99; 1.73 | 0.14 |
| | Brushing vs post-brushing | 0.18 | 0.11; 0.30 | <0.001 |
| Genetic line | R- vs R+ | 1.33 | 1.04; 1.70 | 0.02 |
| Ear posture changes | | | | |
| Phase | Pre- vs brushing | 3.75 | 2.71; 5.17 | <0.001 |
| | Pre- vs post-brushing | 1.50 | 1.1; 2.05 | 0.03 |
| | Brushing vs post-brushing | 0.40 | 0.28; 0.57 | <0.001 |
| Session | 3 vs 1 | 0.63 | 0.45; 0.88 | 0.007 |
| | 1 and 3 vs 2 | 1.09 | 0.82; 1.44 | 0.565 |
| Genetic line | R- vs R+ | 0.73 | 0.56; 0.96 | 0.02 |

In Figure 9, R- sheep expressed raised up ear postures for longer than R+ sheep, which was not found for asymmetric and horizontal postures. In addition, session 3 was characterized by low expression of horizontal ear postures in all phases (FIGURE 9).

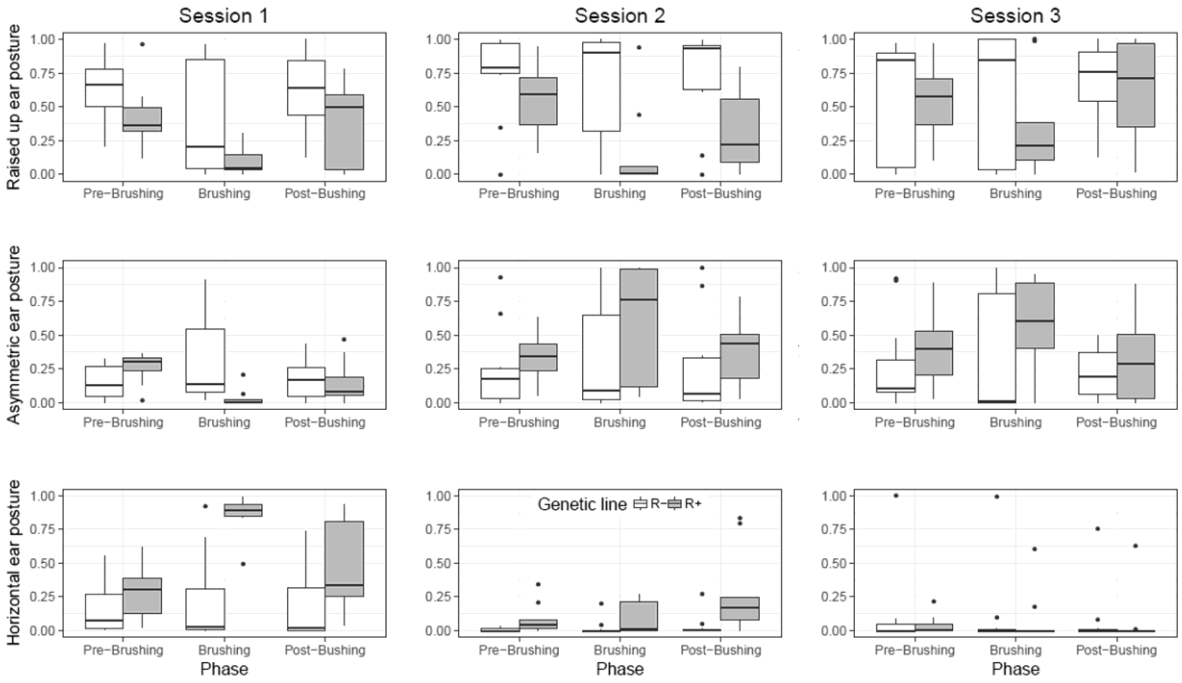


FIGURE 9 - RAISED UP, ASYMMETRIC AND HORIZONTAL EAR POSTURES RECORDED IN 11 LOWLY (R-) AND 9 HIGHLY (R+) REACTIVE EWES, ASSESSED IN PRE-, DURING AND POST-BRUSHING PHASES, IN SESSIONS 1, 2 AND 3

The results for asymmetric and horizontal ear postures showed that reactivity was altered by social separation; here, only the effect of interaction between session and genetic line was significant over such variables ($P<0.05$) (TABLE 8). Among R- sheep, no significant difference was found when the interaction between session and genetic line was considered ($P>0.05$) (TABLE 8). However, R+ sheep expressed asymmetric ear postures for longer in session 3 than in session 1 ($P<0.001$) and in session 2 than in sessions 1 and 3 ($P<0.01$) (TABLE 8). The proportion of time spent on horizontal ears among R- sheep was higher in sessions 1 and 3, than in session 2 ($P<0.001$); among R+ sheep, higher proportion of horizontal ear postures was noted in session 1 than in 3 ($P<0.001$) (TABLE 8). Curiously, for raised up ear postures, the interaction between session and genetic line was not significant ($P>0.05$), but the main effects of phase ($P<0.01$) and genetic line were significant ($P<0.001$) (TABLE 8). Ewes spent more time performing raised up ears pre- ($P<0.01$) and post- ($P=0.01$) than during brushing (TABLE 8); R- sheep expressed such posture for longer than R+ sheep ($P<0.001$) (TABLE 8).

TABLE 8 - ODDS RATIO (OR) FOR THE PROPORTION OF TIME SPENT ON RAISED UP, ASYMMETRIC AND HORIZONTAL EAR POSTURES, IN 20 ROMANE EWES, CONSIDERING THE SIGNIFICANT EFFECTS OF PHASE (PRE-, BRUSHING AND POST-BRUSHING), GENETIC LINE (R- AND R+) AND INTERACTION BETWEEN SESSION (1, 2 AND 3) AND GENETIC LINE

| Variable/Effect | Comparison | OR | CI | P |
|---|---|---|---|---|
| Raised up | | | | |
| Phase | Pre- vs brushing | 2.33 | 1.35; 4.03 | 0.007 |
| | Pre- vs post-brushing | 1.07 | 0.66; 1.73 | 0.96 |
| | Brushing vs post-brushing | 0.46 | 0.26; 0.79 | 0.01 |
| Genetic line | R- vs R+ | 2.85 | 1.87; 4.35 | <0.001 |
| Asymmetric | | | | |
| Session vs genetic line | 3 vs 1 R- | 1.45 | 0.71; 2.94 | 0.31 |
| | 3 vs 1 R+ | 4.30 | 2.17; 8.51 | <0.001 |
| | 1 and 3 vs 2 R- | 0.84 | 0.43; 1.63 | 0.61 |
| | 1 and 3 vs 2 R+ | 0.44 | 0.25; 0.77 | 0.004 |
| Horizontal | | | | |
| Session vs genetic line | 3 vs 1 R- | 0.46 | 0.14; 1.55 | 0.21 |
| | 3 vs 1 R+ | 0.06 | 0.02; 0.17 | <0.001 |
| | 1 and 3 vs 2 R- | 9.01 | 2.80; 29.03 | <0.001 |
| | 1 and 3 vs 2 R+ | 1.95 | 0.90; 4.23 | 0.09 |

Figure 10 shows that, in general, R+ sheep performed closed and half-closed eyes, tail wagging and eating and ruminating for longer, when compared to R- sheep; the median for such behaviours in R- sheep was equal to 0 mainly in pre- and post-brushing phases, in all sessions.

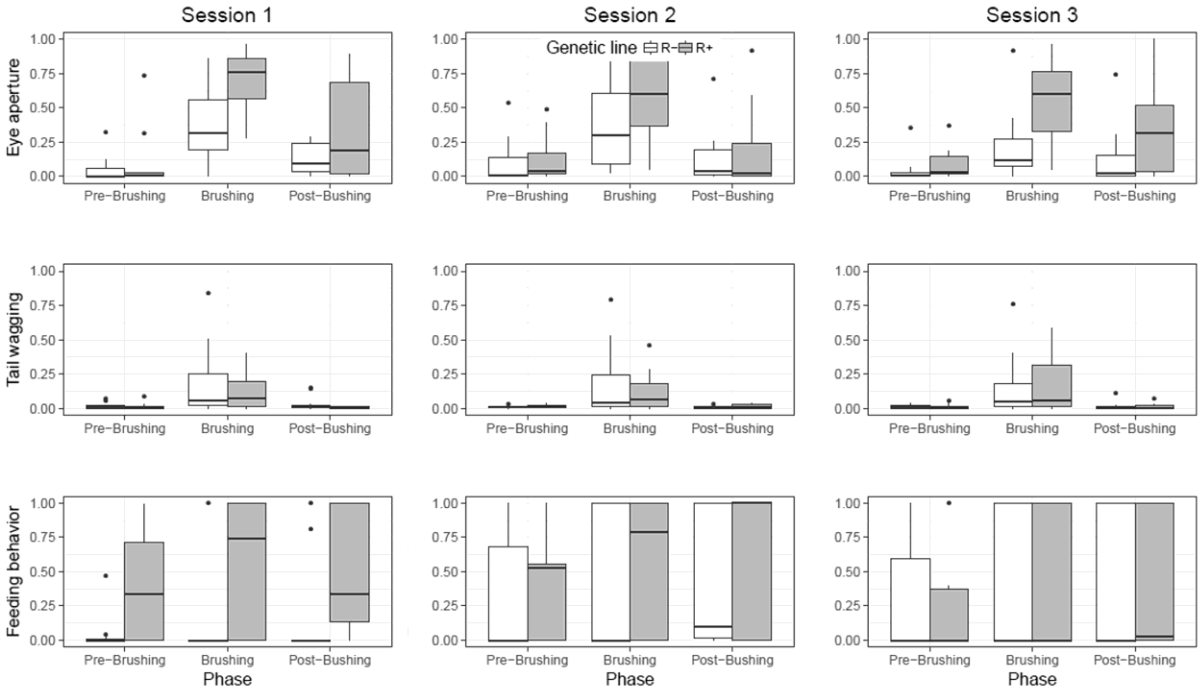


FIGURE 10 - EYE APERTURE, TAIL WAGGING AND FEEDING BEHAVIOUR RECORDED IN 11 LOWLY (R-) AND 9 HIGHLY (R+) REACTIVE EWES, ASSESSED IN PRE-, DURING AND POST-BRUSHING PHASES, IN SESSIONS 1, 2 AND 3

The findings for feeding behavior also suggested that reactivity was influenced by social separation, due to a significant interaction between session and genetic line ($P<0.05$) (TABLE 9). The proportion of time that R-sheep spent eating and ruminating was, on average, higher in session 3 than in session 1 ($P<0.05$) (TABLE 9). For R+ sheep, no significant differences were found ($P>0.05$) (TABLE 9). For eye aperture and tail wagging, there was no significant effect of session ($P>0.05$), but the main effect of phase was significant over both variables ($P<0.001$) (TABLE 9). The proportion of time with closed and half-closed was higher post- than pre-brushing ($P<0.001$), during than pre-brushing ($P<0.001$) and during than post-brushing ($P<0.001$) (TABLE 9). The proportion of time spent on tail wagging was higher during than pre- ($P <0.001$) and post-brushing ($P<0.001$) (TABLE 9); there was no significant difference between pre- and post-brushing phases ($P>0.05$) (TABLE 9). The main effect of genetic line was also significant on eye aperture ($P<0.001$); ewes belonging to the R+ treatment showed longer duration of closed and half-closed eyes than R- ewes ($P<0.001$) (TABLE 9).

TABLE 9 - ODDS RATIO (OR) FOR THE PROPORTION OF TIME SPENT ON EYE APERTURE, TAIL WAGGING AND FEEDING BEHAVIOUR, IN 20 ROMANE EWES, CONSIDERING THE SIGNIFICANT EFFECTS OF PHASE (PRE-, BRUSHING AND POST-BRUSHING), GENETIC LINE (R- AND R+) AND INTERACTION BETWEEN SESSION (1, 2 AND 3) AND GENETIC LINE

| Variable/Effect | Comparison | OR | CI | P |
|---|---|---|---|---|
| Eye aperture | | | | |
| Phase | Pre- vs brushing | 0.11 | 0.06; 0.19 | <0.001 |
| | Pre- vs post-brushing | 0.33 | 0.17; 0.62 | <0.001 |
| | Brushing vs post-brushing | 3.04 | 1.79; 5.16 | <0.001 |
| Genetic line | R- vs R+ | 0.37 | 0.23; 0.59 | <0.001 |
| Tail wagging | | | | |
| Phase | Pre- vs brushing | 0.07 | 0.04; 0.12 | <0.001 |
| | Pre- vs post-brushing | 0.91 | 0.50; 1.66 | 0.95 |
| | Brushing vs post-brushing | 12.33 | 6.45; 23.56 | <0.001 |
| Feeding behaviour | | | | |
| Session vs genetic line | 3 vs 1 R- | 4.78 | 1.36; 16.74 | 0.02 |
| | 3 vs 1 R+ | 0.56 | 0.21; 1.49 | 0.24 |
| | 1 and 3 vs 2 R- | 0.43 | 0.17; 1.10 | 0.07 |
| | 1 and 3 vs 2 R+ | 0.71 | 0.31; 1.61 | 0.41 |

### 4.3.2 Cardiac responses

We observed that HR was higher in R- sheep in sessions 2 and 3, mainly during and post-brushing (FIGURE 11). Similar results can be noted for RMSSD/SDNN rate, in all sessions (FIGURE 11). Regarding LF/HF rate, descriptive findings show in general low medians (FIGURE 11).

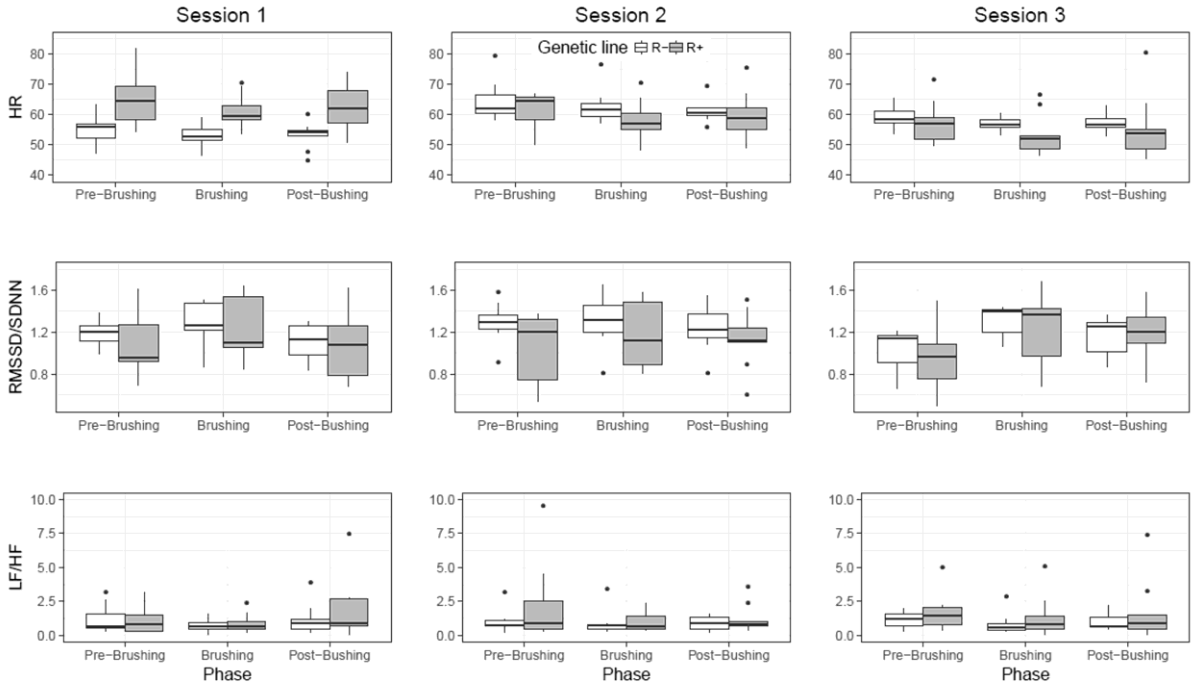


FIGURE 11 - HEART RATE (HR), RMSSD/SDNN AND LF/HF RECORDED IN 11 LOWLY (R-) AND 9 HIGHLY (R+) REACTIVE EWES, ASSESSED IN PRE-, DURING AND POST-BRUSHING PHASES, IN SESSIONS 1, 2 AND 3

Significant effects of interaction between session and genetic line (P<0.0001) and phase and genetic line (P=0.01) over HR were found (TABLE 10). Ewes belonging to the R- line had, on average, more BPM in session 2 than in sessions 1 and 3 (P<0.001) (TABLE 10). In session 3, R- ewes showed, on average, more BPM than in session 1 (P=0.001) (TABLE 10). On the opposite, no significant difference of HR in sessions 1 and 3 in relation to session 2 was noted for R+ sheep (P>0.05) (TABLE 10); sheep belonging to R+ line showed, on average, more BPM in session 1 than in session 3 (P<0.001) (TABLE 10). When the effect of interaction between phase and genetic line was considered, we noted that ewes belonging to the R- line showed more BPM pre- than during (P<0.001) and post-brushing (P=0.003) (TABLE 10). Similar results were noted for R+ ewes; they had more BPM pre- than during (P<0.0001) and after brushing (P<0.01), in addition to less BPM during than post-brushing (P<0.001) (TABLE 10). Regarding RMSSD and SDNN separately, no significant effect was observed (P>0.05), but the effect of interaction between session and phase was significant on RMSSD/SDNN ratio (P<0.01). In session 1, RMSSD/SDNN ratio was higher during than pre- (P=0.05) and post-brushing (P<0.01) (TABLE 10). In session 2, no significant difference was found for RMSSD/SDNN (TABLE 10). In session 3, RMSSD/SDNN ratio was higher during

than pre-brushing (P<0.001); higher in post- than in pre-brushing phase (P<0.01) and higher during than post-brushing (P<0.05) (TABLE 10). The effect of phase was significant on LF/HF ratio (P<0.01). Sheep showed lower LF/HF ratio during than pre- (P<0.01) and post-brushing (P=0.01) (TABLE 10).

TABLE 10 - MEAN DIFFERENCES (MD) FOR HEART RATE AND RMSSD/SDNN, AND RATIO OF MEANS (ROM) FOR LF/HF, IN 20 ROMANE EWES, CONSIDERING THE SIGNIFICANT INTERACTIONS BETWEEN SESSION (1, 2 AND 3) AND GENETIC LINE (R- AND R+), PHASE (PRE-, BRUSHING AND POST-BRUSHING) AND GENETIC LINE, SESSION AND PHASE, AND THE MAIN EFFECT OF PHASE

| Variable/Effect | Comparison | MD/RoM | CI | P |
|---|---|---|---|---|
| Heart rate | | | | |
| Session vs genetic line | 3 vs 1 R- | 4.09 | 1.64; -6.53 | 0.001 |
| | 3 vs 1 R+ | -7.20 | 4.50; 9.90 | <0.001 |
| | 1 and 3 vs 2 R- | -7.51 | -5.30; -9.72 | <0.001 |
| | 1 and 3 vs 2 R+ | -1.21 | 1.12; 3.55 | 0.31 |
| Phase vs genetic line | Pre- vs brushing R- | 1.93 | 0.92; 2.94 | <0.001 |
| | Pre- vs post-brushing R- | 1.67 | 0.67; 2.68 | 0.004 |
| | Brushing vs post-brushing R- | -0.25 | -1.26; 0.75 | 0.87 |
| | Pre- vs brushing R+ | 3.92 | 2.83; 5.02 | <0.001 |
| | Pre- vs post-brushing R+ | 1.86 | 0.76; 2.95 | 0.003 |
| | Brushing vs post-brushing R+ | -2.07 | -3.16; -0.97 | <0.001 |
| RMSSD/SDNN | | | | |
| Session vs phase | Session 1 pre- vs brushing | -0.19 | -0.20; -0.01 | 0.05 |
| | Session 1 pre- vs post-brushing | 0.04 | -0.05; 0.14 | 0.62 |
| | Session 1 brushing vs post-brushing | 0.15 | 0.06; 0.25 | 0.005 |
| | Session 2 pre- vs brushing | -0.07 | -0.17; 0.02 | 0.28 |
| | Session 2 pre- vs post-brushing | -0.02 | -0.11; 0.08 | 0.92 |
| | Session 2 brushing vs post-brushing | 0.06 | -0.04; 0.15 | 0.49 |
| | Session 3 pre- vs brushing | -0.27 | -0.36; -0.17 | <0.001 |
| | Session 3 pre- vs post-brushing | -0.14 | -0.24; -0.05 | 0.008 |
| | Session 3 brushing vs post-brushing | 0.12 | 0.03; 0.21 | 0.03 |
| LF/HF* | | | | |
| Phase | Pre- vs brushing | 1.39 | 1.14; 1.69 | 0.003 |
| | Pre- vs post-brushing | 1.05 | 0.86; 1.28 | 0.87 |
| | Brushing vs post-brushing | -0.76 | 0.62; 0.92 | 0.01 |

* LOG TRANSFORMED

## 4.4 DISCUSSION

Our results indicated that social separation promoted in session 2 might not have affected sheep responses. Temporary separation might not have affected the expression of ear posture changes in session 3, when the animals seemed to be

more relaxed than in session 1. Cardiac data showed a similar pattern: in sessions 1 and 3, the RMSSD/SDNN ratio was higher mainly during brushing, and in session 3, the RMSSD/SDNN ratio was higher after than pre-brushing. The absence of significant changes across phases in session 2 suggests that sheep might not have responded to brushing, according to ear posture changes and RMSSD/SDNN data, and in sessions 1 and 3, the consummatory and post-consummatory phases of brushing elicited parasympathetic activation in sheep.

Highly and lowly reactive sheep might have perceived social separation differently, according to ear postures and HR data, but not for feeding data. Highly reactive sheep expressed asymmetric ear postures for longer in session 2 than in sessions 1 and 3, and in session 3 than in session 1. For R- sheep, no significant results were found. As asymmetric ears seem to be related to sudden, surprising events (BOISSY et al., 2011), our hypothesis that R+ sheep, after being separated from group members, express such posture for longer was confirmed. The fact that asymmetric ears were also expressed for longer in session 3 might indicate that the temporary separation created in session 2 influenced the last session. The findings point to the influence of emotional reactivity on the expression of asymmetric ear postures in R+ sheep. In addition, R- ewes expressed horizontal ear postures for longer in sessions 1 and 3 than in session 2, and R+ ewes, in session 1 than in session 3, suggesting that session 2 had a negative effect on both lines and it might have had a carry-over effect over session 3. It is reported that horizontal ear postures are frequently performed in response to positive events (COULON et al., 2015). Additionally, decreases in duration of horizontal ear postures may also be sensitive indicators in the assessment of negative or less positive events, as social separation.

Interestingly, R- spent more time eating and ruminating in session 3 than in session 1. According to the findings, the effect of temporary separation in session 2 might not have influenced session 3 in R- ewes, or R- ewes seemed to be less sensitive to changes in feeding behaviour concerning temporary separation. Rumination is expressed in response to positive states (VÖGELI et al., 2015), so longer duration of such behaviour in session 1 as compared to session 2 and 3 would be expected. Further research is encouraged to better understand the relation between food ingestion and rumination and emotional reactivity.

Ewes in the R- group had lower HR in sessions 1 and 3 than in session 2. When sessions 1 and 3 were compared, R- sheep had higher HR in session 3. Such

results show that brushing during temporary separation had a less positive or an explicit negative effect on R- sheep, and that session 2 had a residual negative effect on the cardiac responses of R- sheep in session 3. Among R+ sheep, lower HR was found in session 3 than in session 1, and no significant result was found for session 2. It was also expected that the presence of a barrier during session 2 would activate the sympathetic system of R+ sheep, with a possible influence on the session 3. A possible explanation is that brushing might have elicited a more positive, rewarding state on R+ ewes than on R- ewes, regardless of the presence of the second barrier, as noted for some behavioural results. Consequently, the use of a physical barrier might not have influenced the responses of R+ sheep. Since differences were found, our results indicate that cardiac responses may be related to emotionality. This relationship has been described in other animal species, as reported by Valance et al. (2007), who found that selection on tonic immobility duration in quail might have affected sympathovagal control.

The findings also showed that sheep experienced a positive, relaxing state in response to gentle tactile stimulation, as they performed less body postures, head orientation and ear postures changes, more closed and half-closed eyes and tail wagging, and showed lower HR and LF/HF ratio and higher RMSSD/SDNN when brushed, corroborating literature findings (HARGREAVES; HUTSON, 1990; AURELI; PRESTON; DE WAAL, 1999; MOHR; LANGBEIN; NURNBERG, 2002; WAIBLINGER et al., 2004; SANDEM; BRAASTAD; BAKKEN, 2006; REEFMANN et al., 2009; REEFMANN; WECHSLER; GYGAX, 2009; ZEBUNKE; PUPPE; LANGBEIN, 2013; COULON et al., 2015; LAMBERT; CARDER, 2017; TAMIOSO et al., 2017). Anticipatory behaviour, expressed by increased activity, and activation of the sympathetic tone before being brushed were found through more body posture, head orientation and ear posture changes and longer duration of raised up ear postures, in addition to higher HR and lower RMSSD/SDNN; such responses might be associated with the anticipation of positive situations in domestic animals (ZEBUNKE et al., 2011; GYGAX et al., 2013; ANDERSON et al., 2015). Increased activity, attention and sympathetic activation in the phase following brushing was noted by higher performance of head orientation and ear posture changes, raised up ear postures and LF/HR ratio, and might be due to possible appreciation of brushing and expectation to be brushed for longer. For example, the maintenance of raised up ear postures might be related to increased attention toward a given situation of positive

valence (MOE et al., 2006). So far there is limited data on the pre-, consummatory and post-consummatory phases of positive stimuli in animals. It seems that consummatory phases of positive events of lower arousal produce relaxing responses, as parasympathetically dominated power spectrum. In addition, R+ and R- sheep responded differently to brushing in relation to some behavioural responses. In general, the results suggest that R+ sheep might have had a more positive, relaxing response to brushing.

The relative distance between the brushed animal and pen mates in session 2 did not seem to influence the relaxing responses induced by brushing, according to the results for ear posture changes and RMSSD/SDNN ratio. On the opposite, an important association between social reactivity and social context was observed when R+ and R- sheep were assessed throughout the sessions: asymmetric ear postures pointed to a negative influence of social separation over R+ sheep responses; horizontal ear postures showed that the presence of physical barriers affected both lines either in session 2 or acted as a carry-over effect in session 3; and HR data showed that separation from group members altered emotional reactivity of R- sheep. Divergent findings might be related to the fact that the animals were not adapted to the new scenario of separation, which might have resulted in generalized responses. The results also pointed to an overall important effect of phase over sheep behavioural and cardiac responses. Pre-brushing was characterized by anticipation, sympathetic activation, brushing by satisfaction, parasympathetic activation, and post-brushing, by expectation, sympathetic activation.

## 4.5 CONCLUSION

The indicators suggested that social separation influenced sheep responses, and social separation had a less negative effect over R+ sheep. Divergent results on the studied indicators point to the necessity of further studies in order to confirm if the responses observed were specific to social separation or if other stimuli that altered a known situation may elicit similar results. Further investigations might also collaborate to a better understanding of the relationship between the emotional reactivity and the emotional context, here through the social context, in which positive handling may be performed. Our findings might help in developing strategies to

promote positive welfare to animals, by using brushing to enrich the housing conditions on farms in a manner that considers the relevant effects of social conditions.

## 4.6 ACKNOWLEDGMENTS

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# 5. ATTITUDES OF SOUTH BRAZILIAN SHEEP FARMERS TO ANIMAL WELFARE AND SENTIENCE

## RESUMO

Objetivou-se estudar as atitudes de 148 ovinocultores do sul do Brasil em relação a bem-estar e senciência animal. A maioria dos produtores (73,0%) conhecia bem-estar animal superficialmente. Produtores que trabalhavam mais tempo na indústria ovina e que criavam ovinos para fins comerciais mencionaram mais comumente que tinham conhecimento sobre bem-estar animal (P<0,05). Termos relacionados à liberdade de fome, sede e desnutrição foram os mais usados para definir bem-estar animal, citado 24,9% das vezes pelos produtores. A maioria afirmou que seus animais possuem níveis adequados de bem-estar (93,2%), especialmente produtores que mantinham rebanhos maiores (P<0,05). No entanto, muitos respondentes acreditavam que o bem-estar dos ovinos poderia ser melhorado em suas fazendas (71,6%), principalmente produtores com menos experiência na indústria ovina (P<0,01). Altos escores de senciência foram atribuídos a ovinos por produtores com contato frequente com seus animais (P<0,05). De acordo com os produtores, a castração causa os maiores níveis de sofrimento aos ovinos (32,4%) e a tosquia, os menores (50,0%). O conhecimento de produtores do sul do Brasil sobre bem-estar animal, as atitudes em relação à senciência e o reconhecimento do sofrimento precisam ser melhorados.

Palavras-chave: Bem-estar animal. Emoções. Opinião. Percepção.

## ABSTRACT

We investigated self-reported attitudes of 148 South Brazilian sheep farmers to animal welfare and sentience. Many farmers (73.0%) knew animal welfare superficially. Farmers that worked for longer in the sheep industry and that raised sheep for commercial purposes mentioned more commonly that they had knowledge about animal welfare ($P<0.05$). Terms related to freedom from hunger, thirst and malnutrition were the most used to define animal welfare, cited 24.9% of the times. The majority claimed that their animals experience good welfare (93.2%), especially farmers that kept bigger flocks ($P<0.05$). However, many respondents believed that sheep welfare could be improved on their farms (71.6%), mainly farmers with less experience in the sheep industry ($P<0.01$). High scores of sentience were attributed to sheep by farmers with frequent contact with their animals ($P<0.05$). According to the farmers, castration causes the highest levels of suffering to sheep (32.4%) and shearing, the lowest (50.0%). South Brazilian farmer knowledge about animal welfare, attitudes to sentience and recognition of suffering need improvement.


Keywords: Animal Welfare. Emotions. Opinion. Perception.

## 5.1 INTRODUCTION

Investigations about the perception and attitude of farmers to animal welfare have been reported in the literature and revealed important views about the subject. Te Velde, Aarts and van Woerkum (2002) observed that farmers showed some knowledge about policies and regulation in animal welfare, although they were not prone to alternative ways of farming with special attention to animal welfare. Farmers' perceptions and attitudes may also be directly and strongly associated with their behaviour towards animals and subsequent behaviour of the animals and their production (HEMSWORTH et al., 2002).

Attribution of sentience to animals may also affect human-animal relationships and attitudes toward animals. A positive relation between the recognition of an animal mind, i.e., the extent to which animals have awareness, thoughts and emotions, and animal welfare has been reported (MORRIS; KNIGHT; LESLEY, 2012). Hills (1993) noted that Australian farmers supported human dominance over animals and showed lower levels of empathy towards animals that had lower instrumental significance for them, when compared to animal rights supporters and members of the urban public. As attitudes are learnt and changed with experience (AJZEN, 2005), we believe that positive attitudes to welfare and sentience in animals may subsequently influence the behaviour of farmers toward the animals. Thus, our work aimed to investigate the attitudes of sheep farmers to animal welfare and sentience.

## 5.2 MATERIAL AND METHODS

The total number of sheep in Brazil was estimated at 18 410 551 animals, being the Northeast region the greatest sheep producer (60.6%), followed by the South region (26.5%) (IBGE, 2015). In the State of Parana, the total number of sheep was estimated at 614 749 animals, i.e. 3.3% of the total number of sheep in Brazil (IBGE, 2015). In Brazil, the number of sheep farms was estimated at 438 623, from a total of 365 754 owners (IBGE, 2006). In the State of Parana, there were 17 434 sheep farms and 15 960 owners (IBGE, 2006). Data provided by the Sheep Breeders Associations of Parana contained 312 contacts of sheep farmers. From these, 78 did not raise sheep in Paraná anymore, 48 could not be contacted through telephone or

email, 24 did not want to participate in the survey and 14 contacts were duplicated from the same farmers. In total, 148 sheep farmers were successfully interviewed by telephone. The interviews comprised a sample with a confidence level of 95%. The study was conducted from December 2014 to May 2015. The study was approved by the Human Research Ethics Committee of the Federal University of Paraná (CEP/SCS/UFPR), under protocol number 814 835/2014 (ANNEX III).

The questionnaire consisted of 19 questions; the first section was composed of demographic and general data on gender, age, education, flock size, sheep breed, farming system, experience in the sheep industry, contact with sheep and purpose of production (n=9). The interview then proceeded with a section on questions about animal welfare (n=4) (TABLE 11). Then, the farmers were asked to respond questions about sheep welfare and sentience (n=5) (TABLE 11). The last section covered one question on levels of emotions in different species of animals (TABLE 11). The interviews lasted on average 30 minutes, ranging from 20 to 40 minutes.

TABLE 11 - MAIN QUESTIONS APPLIED DURING TELEPHONE INTERVIEWS WITH 148 SHEEP FARMERS ABOUT ANIMAL WELFARE AND SENTIENCE, WITH SPECIAL ATTENTION TO SHEEP, IN PARANA, SOUTH OF BRAZIL, DECEMBER 2014 TO MAY 2015

| Questions | Content | Options of answers |
|---|---|---|
| Q01 | Have you ever heard of animal welfare? | Yes, I know what animal welfare is; Yes, I know the subject superficially; No, I have never heard of animal welfare. |
| Q02 | If yes, what do you think animal welfare consists of? | Open question. |
| Q03 | What are the most important aspects of animal farming that contribute to good animal welfare? | Open question. |
| Q04 | Do you think welfare is taken into consideration for farm animals? | Yes, fully; Yes, most of the times; Yes, half of the times; Yes, a few times; No, never; I do not know. |
| Q05 | Do you think that sheep on your farm have a good level of welfare? | Yes; No; I do not know. |
| Q06 | Do you think that the level of welfare could be improved? | Yes; No; I do not know. |
| Q07 | What are the welfare aspects you find most difficult to improve on your farm? | Open question. |

| | | |
|---|---|---|
| Q08 | In a scale from 1 to 5, please select the rating that best describes your opinion:<br>Sheep that are healthy and grow well have their welfare guaranteed.<br>Sheep that are raised indoors, under intensive management systems, have low levels of welfare.<br>Sheep clearly distinguish between handler and other people.<br>Sheep are capable of feeling emotions, such as fear, and happiness, in addition to suffering.<br>Sheep clearly express how they feel, that is why it is easy to identify if they are in positive or negative situations. | 1; 2 ; 3; 4; 5; I do not know<br>1 strongly disagree; 2 disagree; 3 neutral/unsure; 4 agree; 5 strongly agree. |
| Q09 | In a scale from 1 to 5, classify the management procedures that are frequently performed on sheep farms according to your perception on sheep suffering: identification, castration, tail docking, shearing, reproductive techniques and weaning. | 1; 2 ; 3; 4; 5; I do not know<br>1 no suffering; 2 mild suffering; 3 moderate suffering; 4 severe suffering; 5 very severe suffering. |
| Q10 | In a scale from 1 to 5, classify the ability of each animal to feel emotions: pigeon, butterfly, human baby, rat, dog, chicken, fish, sheep, cattle, cockroach and wolf. | 1; 2 ; 3; 4; 5; I do not know<br>1 the animal does not feel emotions; 5 the animal certainly feels emotions; intermediate values are equivalent to a growing capacity to feel emotions. |

Descriptive analysis was conducted in Excel, where absolute and relative frequencies were plotted against some variables of interest, as gender, education, flock size, experience in the sheep industry, contact with sheep and purpose of production. After, data were analyzed using Statistica Software, version 7.0. Chi-square or Fisher's exact tests were used to verify significant associations between the variables of interest for each question. Statistical significance level was set to $P<0.05$. Open questions on the definition of animal welfare, the most important aspects of animal farming that contribute to good animal welfare and the most difficult welfare aspects to improve on the farm were categorized according to the perspective of the Five Freedoms (FAWC, 1992). For example, "sheep must be fed and have water *ad libitum*, and veterinary care", from a male farmer aged at 52 years-old, was categorized in both freedom from hunger, thirst and malnutrition and

freedom from pain, injury and disease. Answers that could not be included in the Five Freedoms, as “the animals must be far from the human being”, from a male farmer aged at 49 years-old, were categorized as Other.

## 5.3 RESULTS AND DISCUSSION

### 5.3.1 Demographic data

The majority (84.5%; 125/148) was male farmers and 15.5%, female farmers (23/148). The age of the study population ranged from 40-49 (27.7%; 41/148), 50-59 (25.7%; 38/148) and 60 years-old or more (22.9%; 34/148); most farmers (41.9%, 62/148) completed higher education; flock sizes were mainly less than 100 sheep (40.5%, 60/148). Sheep breeds raised on the farms were mainly Texel (41.2%, 61/148), Santa Inês (22.3%, 33/148) and Île-de-France (16.2%, 24/148). One hundred and twenty eight farmers (86.5%) kept their sheep in semi-intensive systems. Most of the farmers (35.9%, 53/148) had more than 20 years of experience with sheep. Eighty three (56.1%) respondents had daily contact with their animals. Sixty-five (43.9%) and 61 (41.2%) farmers raised sheep for commercial purposes and for both own consumption and commercial purposes, respectively.

### 5.3.2 Animal welfare

Most of the farmers responded that they had superficial knowledge about animal welfare (73.0%; 103/148). Farmers who worked for longer in the sheep industry (P=0.045) and those who raised sheep for commercial purposes (P=0.0035) had more knowledge about animal welfare. Therefore, we detected that knowledge on animal welfare was higher among farmers that probably had more monetary gains, the opposite as reported by Kiliç and Bozkurt (2013). However, further studies are necessary to understand the relation between knowledge of animal welfare and different factors of sheep farming.

The respondents that claimed to know animal welfare, defined the subject using terms related to freedom from hunger, thirst and malnutrition (24.9%; 71/285), freedom from pain, injury and disease (23.2%; 66/285) and freedom from discomfort (22.1%; 63/285). Te Velde, Aarts and van Woerkum (2002) observed that farmers

defined animal welfare in terms of physical health, i.e., if an animal eats well, it is healthy and it has a good level of welfare. Farmers answered that the most important aspects of animal farming that contribute to good animal welfare comprised freedom from hunger, thirst and malnutrition (33.4%; 109/326) and freedom from discomfort (24.8%; 81/326). Vanhonacker et al. (2008) also found that dimensions associated with feed and water and animal health were considered to be the most important by citizens and farmers when obtaining a suitable level of farm animal welfare. Our study shows that the perception of important aspects of animal farming may be associated with the terms used by the farmers to define animal welfare.

When asked if welfare is considered for livestock species, different responses were given: for 27.0% (40/148) of the farmers, animal welfare is considered a few times, for 24.3% (36/148) animal welfare is considered half of the times and for 20.3% (30/148), it is never taken into consideration. The plurality of opinions given by farmers did not allow any guidance on their attitudes. It appears that the superficial knowledge about animal welfare contributes to the limited view on how it may be applied to the livestock scenario.

### 5.3.3 Sheep welfare and sentience

One hundred thirty-eight (93.2%) believed that sheep on their farms had a good level of welfare, as also observed by farmers in a study by Te Velde, Aarts and van Woerkum (2002). We also noted that the bigger the flock size, the higher was the level of welfare attributed by farmers to sheep on their farms (P=0.04). Kiliç and Bozkurt (2013), however, found a negative correlation between animal welfare and farm size. As animal welfare was defined mostly in terms of health and nutrition by the farmers in our study, it is evident that farmers' perception of animal welfare was deficient and, thus, it is impossible to estimate whether sheep experienced adequate degrees of welfare. A total of 71.6% (106/148) responded that the level of welfare of their animals could be improved. Higher number of farmers with less experience in sheep industry responded that animal welfare could be improved on their farms (P=0.008), pointing to the recognition of better levels of farm animal welfare by such respondents.

For the farmers, the most difficult aspects to be improved were related to the freedom from discomfort (28.3%; 47/166) and freedom from hunger, thirst and

malnutrition (27.1%; 45/166). Australian sheep farmers also reported that poor nutrition was considered the biggest welfare issue on their farms, followed by fly strike, drinking water availability, intestinal parasites, among others (PHILLIPS; PHILLIPS, 2010). Experience in the sheep industry was associated with specific welfare aspects to be improved; for example, the majority of respondents who included aspects related to freedom from pain, injury and disease (13.3%; 22/166) were farmers with less experience (P=0.04), which suggests higher perception of presence of diseases and pain in sheep by farmers that work with sheep for shorter periods of time. On the opposite, more farmers with more experience reported that no welfare aspect needed to be improved (P=0.0095). Such result indicates that farmers with more experience may not perceive welfare problems, which requires special attention.

Seventy-five (50.7%) and 58 (39.2%) farmers agreed and strongly agreed, respectively, that sheep that are healthy and grow well have their welfare guaranteed. It is interesting to note that agreement with this statement was mainly given by farmers that raised sheep for commercial purposes (P=0.02). Phillips and Phillips (2010) also found that several sheep farmers related animal welfare to productivity, mentioning that happy sheep produce more. Results from both studies reinforce a recurring idea that welfare is mainly related to physical health, fast growth and food conversion; thus, it is necessary that the farmers be clearly explained that animal welfare comprises different factors.

When asked if sheep that are raised indoors, under intensive management systems, have low levels of welfare, different answers were observed: 45.9% (68/148) agreed, 20.9% (31/148) disagreed and 16.2% (24/148) were unsure. It is known that extensive farming provides the animals the opportunity to engage in natural behaviour, despite exposing them to more environmental challenges; on the opposite, confinement systems enable farmers to protect their animals from predation, some parasites and harsh weather. It seems necessary to promote thinking on different aspects and restrictions of indoor and outdoor systems in order to provide improved levels of welfare to the animals.

On sheep sentience, 78 (52.7%) and 54 (36.5%) agreed and strongly agreed, respectively, that sheep clearly distinguish between handler and other people. A higher level of perception regarding the cognitive abilities of sheep was scored by farmers that had frequent contact with their animals (P=0.003). The literature reports

evidences of sheep complex recognition skills (KENDRICK et al., 2001). The majority of farmers agreed (54.7%; 81/148) and strongly agreed (33.8%; 50/148) that sheep are able to feel emotions; 6.8% (10/148) of the farmers were unsure.

A total of 51.4% (76/148) and 29.7% (44/148) agreed and strongly agreed, respectively, that sheep clearly express how they feel, that is why it is easy to identify if they are in positive or negative situations. Most of Turkish farmers also believed that sheep are sentient beings (KILIÇ; BOZKURT, 2013). Recognition that sheep feel emotions has a very important application, since it may contribute to a positive behaviour of the farmers towards sheep management. Higher scores of perception of sentience were rated by farmers that had frequent contact with sheep (P=0.02). It is documented that familiarity with animals strongly influences people's beliefs about animal sentience (MORRIS; KNIGHT; LESLEY, 2012).

On animal suffering generated by management procedures performed in sheep farming, 39.2% (58/148) and 28.4% (42/148) responded that identification through methods regularly employed, as ear tattooing and tagging, cause mild and moderate suffering to sheep, respectively (FIGURE 12). Welfare problems related to the use of ear tags have been reported in the literature. Edwards and Johnston (1999) examined 1040 sheep ears with tag and found that only the plastic ear tags caused slight to moderate ear damage to approximately 28% of the studied sheep. Alternative methods to metal and plastic tags, as the electronic tags, should be further studied and encouraged as they might cause less suffering to sheep.

Regarding castration, 32.4% (48/148) and 31.8% (47/148) reported that sheep experience severe and very severe levels of suffering, respectively (FIGURE 12). Different methods of castration can be applied, but the most used technique includes the application of an elastrator ring. There is strong evidence that castration through such technique induces acute and chronic pain in lambs. In a review study, Stafford and Mellor (2005) suggested that surgical castration should be preferred to the ring method if management permits, and the use of local anaesthetic should be encouraged.

In relation to tail docking, the majority (31.8%; 47/148) attributed moderate suffering to sheep (FIGURE 12). Suffering caused by castration was rated higher than that caused by tail docking, as also observed by Dwyer (2009) in a study with British sheep farmers. It is clear that tail docking, as well as castration, causes

suffering to the animals involved, so it is necessary to invest in more studies to find other alternatives.

Fifty-one percent (75/148) of farmers responded that sheep do not suffer when sheared (FIGURE 12). Nine farmers mentioned that shearing is beneficial to sheep welfare at the start of hot seasons. However, if not conducted properly, shearing may cause injuries to sheep; some authors also reported that shearing may cause stress to sheep (SANGER; DOYLE; HINCH, 2011). In cases that wool production is not viewed as a financial resource for farmers, raising woolless sheep breeds or genetic selection to reduced fleece may be interesting strategies to be considered.

Reproductive techniques were perceived as attached to different levels of suffering: 24.5% (36/147) attributed moderate suffering, 23.8% (35/147) no suffering and 21.1% (31/147) mild suffering (FIGURE 12). The variety of responses indicates that many farmers did not know about the impact of the techniques on sheep welfare. The use of breeding techniques in sheep industry has grown significantly; however, the impact on animal welfare has not been adequately discussed. Murray and Ward (1993) reviewed the welfare implications of available breeding technologies and concluded that while laparoscopic techniques can be effective in reducing timescales for breeding rates, adverse experiences for females may impact negatively on animal welfare. It seems that more studies are necessary to evaluate the real effect of reproductive techniques on sheep welfare.

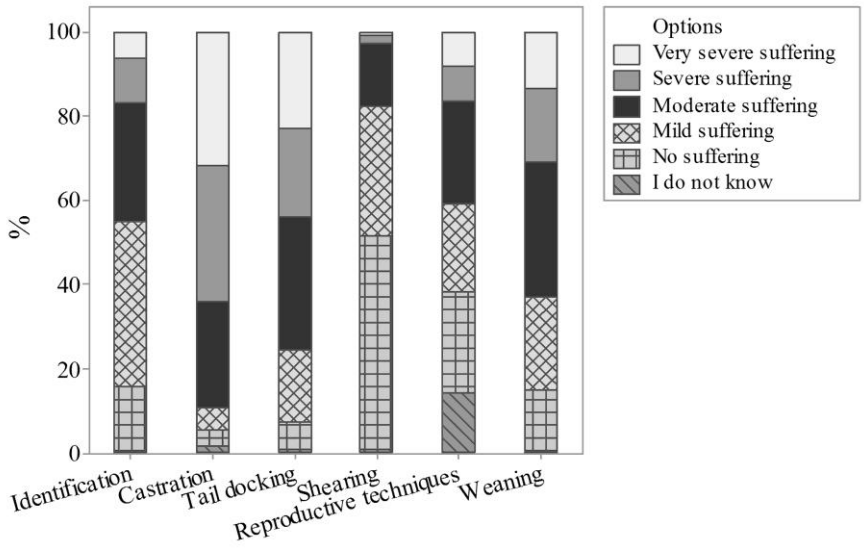


FIGURE 12 - LEVELS OF SUFFERING ATTRIBUTED TO DIFFERENT MANAGEMENT PROCEDURES BY 148 SHEEP FARMERS IN PARANÁ, SOUTH OF BRAZIL, 2014/2015

When questioned about weaning, 31.8% (47/148) responded that sheep suffer moderately (FIGURE 12). Different ages to wean lambs and its behavioural and physiological consequences to ewes and lambs are reported in the literature (SEVI et al., 2003). As weaning is a regular management procedure, it seems mandatory to find new strategies to avoid suffering. Higher level of suffering in weaning was attributed by farmers that had bigger sheep flocks (P=0.01). Farmers that raised sheep for commercial purposes believed that animals experience higher levels of suffering than farmers that raised sheep for their own consumption during weaning (P=0.02). The relation between higher levels of suffering, bigger flocks and commercial view on the production is an interesting result that has not been reported in the literature and demands further investigations.

### 5.3.4 Emotions in different species of animals

The majority of farmers claimed that they did not know about the emotional capacities of pigeon (48.0%; 71/148), butterfly (56.1%; 86/148), fish (29.7%; 44/148) and cockroach (42.6%; 63/148). Uncertain opinions might be considered a negative attitude or feeling towards these animals. The human baby and dog were scored the highest emotional capacities by 92.6% (137/148) and 82.4% (122/148) of farmers, respectively. Thirty-nine (26.4%) scored the highest rate and 32 (21.6%) did not know about sentience of rats. Chicken was rated different levels of sentience abilities: 26.4% (39/148) for the highest level and 25.7% (38/148) for moderate levels of sentience. For both sheep and cattle, similar emotional capacities were observed: 58.1% (86/148) and 53.4% (79/148) of the respondents, respectively, scored the highest level of sentience. It is noteworthy saying that no farmer scored the lowest rate for sheep (i.e. sheep do not experience emotions). Wolves were rated the highest capacity of sentience by 38.5% of the farmers (57/148). A hierarchy of sentience was observed, being the mammals the highest scored animals, followed by birds, pigeons, fish, and invertebrates. Literature data show that humans usually prefer animals that are more phylogenetically, behaviourally or physically close to humans (SERPELL, 2004). Females attributed higher levels of emotions to chicken (P=0.01) and sheep (P=0.03) than male farmers. Women are generally reported to be more sensitive and emphatic towards animals than men (TAYLOR; SIGNAL, 2005). Results may be explained by the fact that the female farmers were probably

responsible for taking care of the animals raised on the farm, and, consequently, they have more familiarity to them. It is the first time that the position of farmers to the sentience of different species is reported, providing additional support for actions aiming to include them in welfare legislations.

## 5.4 CONCLUSION

Our results indicate that Brazilian sheep farmer knowledge of animal welfare, attitudes to sentience and recognition of suffering due to specific procedures need improvement. As arguments for animal protection are founded on evidences and beliefs that animals have emotions, it is hoped that farmers' recognition of sentience helps both modifying practices that generate low welfare and applying legislation to promote sheep welfare.

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# 6. PERCEPTION OF ANIMAL SENTIENCE BY BRAZILIAN AND FRENCH CITIZENS

## RESUMO

Comparou-se a percepção de cidadãos comuns de Curitiba, Brasil (OB) e Clermont-Ferrand, França (OF), assim como OB, médicos veterinários (VB), biólogos (BB) e zootecnistas (AB) de Curitiba, em relação ao bem-estar e senciência animal. Foram também apresentados três vídeos exibindo ovinos em situações positivas e negativas. No total, 1103 respondentes participaram do estudo, sendo 388 OB, 350 OF, 248 VB, 92 BB e 25 AB. Bem-estar animal foi definido pelos participantes por meio de termos principalmente associados à liberdade de medo e distresse. Diferenças significativas entre OB e OF indicaram diferentes percepções de questões de bem-estar animal, principalmente na consideração de bem-estar no cenário produtivo e sofrimento em ovinos. Cidadãos e BB apresentaram percepções semelhantes de bem-estar e senciência animal; VB e AB também apresentaram percepções semelhantes. Mulheres tiveram maior percepção de bem-estar e senciência do que homens. Respondentes mais velhos também mostraram maior conhecimento sobre bem-estar animal e percepção sobre senciência animal. A maioria reconheceu corretamente as situações apresentadas nos vídeos, e os descritores utilizados foram semelhantes entre os respondentes, indicando percepções semelhantes sobre emoções em ovinos. Em geral, OB apresentaram maior percepção de questões de bem-estar animal, em comparação com OF; OB e BB também apresentaram maiores percepções dos assuntos abordados, quando comparados a VB e AB. Esta é a primeira comparação entre respondentes sul-americanos e europeus sobre bem-estar e senciência animal, e os resultados fornecem conhecimentos importantes acerca de diferenças interculturais, os quais podem, por sua vez, subsidiar abordagens mais adequadas e eficientes à melhoria do bem-estar animal de forma adaptada.

Palavras-chave: Atitudes humanas. Bem-estar animal. Emoções em animais. Opinião. Ovinos. Pesquisa de opinião.

## ABSTRACT

We compared the perception of ordinary citizens from Curitiba, Brazil (OB) and Clermont-Ferrand, France (OF), as well as OB, veterinarians (VB), biologists (BB) and animal scientists (AB) from Curitiba, concerning animal welfare and sentience. We also presented videos showing sheep in positive and negative situations. In total, 1103 respondents participated in this study, being 388 OB, 350 OF, 248 VB, 92 BB and 25 AB. Animal welfare was defined by the participants using mainly terms associated with freedom from fear and distress. Significant differences between OB and OF pointed to different perceptions of animal welfare issues, mainly on their consideration of welfare in the livestock scenario and sheep suffering. Citizens and BB had similar perceptions of animal welfare and sentience; VB and AB also showed similar perceptions. Females had higher perception of animal welfare and sentience than males. Older respondents also showed higher knowledge about animal welfare and perception of animal sentience. The majority correctly recognized the situations on the videos, and the descriptors were similar among respondents, suggesting similar perceptions of sheep emotions. In general, OB presented a higher perception of animal welfare, in comparison with OF; OB and BB also showed higher perceptions of the subjects as compared to VB and AB. This is the first comparison between South American and European respondents about animal welfare and sentience, and results provide interesting insights into significant cross-cultural differences, which may in turn support more adequate and efficient approaches to the improvement of animal welfare in a tailored fashion.

Keywords: Human attitudes. Animal welfare. Animal emotions. Opinion. Sheep. Survey.

## 6.1 INTRODUCTION

Scientific studies showing evidences of rich emotional capacities in farm animals contributed to a growing interest in ethical and welfare issues, and such concern influences more and more consumer choices for animal products associated with higher levels of animal welfare and lower levels of suffering. According to Te Velde, Aarts and van Woerkum (2002), perceptions of animal welfare may be related to culture, traditions, beliefs, values and interests. Perceptions and attitudes are also related to the degree of proximity and information about the maintenance conditions of the species with which people interact. Furthermore, the attribution of emotional experiences to animals is directly associated with a positive treatment towards them (KNIGHT et al., 2004; PHILLIPS; MCCULLOCH, 2005). Combined with scientific studies on affective states and cognition in farm animals, the recognition that they are sentient beings may increase the value given to the need for prioritizing their welfare.

Thus, it seems important to understand citizens' perception of animal welfare and sentience, as citizens participate in political processes that may influence or define the conditions domestic animals face throughout their lives. Studies on the perception of professionals who interact with animals also seem essential, as they are directly involved in issues associated with animal welfare and contribute to spread information on animal welfare to several sectors of society, as citizens, consumers, farmers and stock people. The more people attribute emotional capacities to animals, the more they will respect them and the welfare status of the animals will be preserved. In addition, recognizing that animals experience emotions will have relevant consequences as it might contribute to the appreciation of their moral status (GRAY; GRAY; WEGNER, 2007).

By contrast to cattle, pigs and poultry that are intensively managed, sheep are not commonly given significant societal attention for animal welfare, since they are frequently associated with extensive production systems. Such systems convey the idea that the animals are raised in a more natural situation and that, therefore, experience adequate levels of welfare (LAWRENCE; CONINGTON, 2008). However, due to certain potentially harmful management procedures employed in the sheep industry, as well as other practices that have raised attention of the general public, as transport and slaughter, there seems to be a growing awareness and concern about sheep welfare (MIELE, 2016). So far there have been few studies about the society

perception in relation to sheep welfare and sentience (HELESKI; MERTIG; ZANELLA, 2004; GODDARD et al., 2006; KILIÇ; BOZKURT, 2013). In a study with American faculty members, Davis and Cheeke (1998) found that the respondents attributed lower mental abilities to sheep when compared to dogs, cats, and horses, but also pigs and cattle; some participants also mentioned that animals with higher levels of mental abilities would require better care to avoid boredom.

Therefore, our study aimed to describe and compare the perception toward welfare and sentience in animals and more particularly in sheep, between ordinary citizens from Curitiba, Brazil and Clermont-Ferrand, France, as well as ordinary citizens and different professionals from Curitiba who interact with animals.

## 6.2 MATERIAL AND METHODS

Respondents from Curitiba, South of Brazil and Clermont-Ferrand, Center of France, were invited to participate in an online survey on Survio® platform from November 2014 to May 2016, available in their respective languages. In Curitiba, the target respondents were expanded to include four groups: ordinary citizens (OB), veterinarians (VB), biologists (BB) and animal scientists (AB). From a total of 986 respondents in Brazil, 753 were selected, as they lived in Curitiba, Brazil, being 388 OB, 248 VB, 92 BB and 25 AB. In Clermont-Ferrand, only ordinary citizens (OF), i.e. without distinction of socio-professional category, were assessed. A total of 376 respondents participated in the survey in France, and 350 were selected, as they lived in the city of Clermont-Ferrand. In total, responses from 1103 participants were evaluated. The minimum sample in each group of respondent was obtained through a formula for unrestricted random sampling by Schaeffer et al. (1990), according to the population of Curitiba in the 2010 Census and Clermont-Ferrand, in the 2014 Census. For VB, BB and AB, both the Regional Council of Veterinary Medicine and the Regional Council of Biology of the State of Parana provided the number of veterinarians, animal scientists and biologists registered in Curitiba. The survey comprised a sample with a margin of error equal to 5% and confidence level of 95% for each respondent group. The level of significance was set at $P<0.05$. The study was approved by the Human Research Ethics Committee of the Federal University of Paraná (Cométicа - SCS/UFPR), protocol number 814 835/2014 (ANNEX III).

The questionnaire contained 18 open-ended, multiple choices or 5-point Likert-type scale format questions on demographic data, animal welfare in general and sheep welfare and sentience, divided into six sections. Demographic questions, as gender, age and education constituted the first section. The second section comprised four questions about animal welfare in general (Q01-Q04) (TABLE 12). The next section was composed of two questions about proximity to sheep and sheep welfare and sentience (Q05-Q06) (TABLE 12). The fourth section introduced two questions about sheep suffering, through different management procedures that are commonly performed in the sheep production chain. Such questions were presented twice, so that the answers were evaluated according to the respondents' perception when the management procedures were presented without descriptions (identification1, castration1, tail docking1, shearing1, reproductive techniques1 and weaning1) (Q07) and with descriptions of how they are commonly performed (identification2, castration2, tail docking2, shearing2, reproductive techniques2 and weaning2) (Q08) (TABLE 12). The following section was related to sentience in different animal species (Q09) (TABLE 12). The last section covered the perception toward three videos up to 50 seconds showing sheep in situations that elicited different emotional states. The first video showed a female lamb exploring pasture and expressing play behaviour (V1); the second, an isolated female lamb in an unfamiliar pen (V2); and the third video exhibited a male sheep being brushed by a familiar person (V3). Each video was introduced twice: first, the respondents described the emotional state of the animal using three adjectives of their choice (Q10-Q12) and second, they chose three from 10 descriptors with different emotional valences, adapted from the Qualitative Behavior Assessment - QBA® (Q13-Q15) (TABLE 12). Before the beginning of the survey, three experts on animal emotions evaluated the valence of the videos, and they agreed that V1 represented a potentially positive event, V2, a potentially negative event and V3, another potentially positive event. Furthermore, the valence of the event exhibited in each video was supported by scientific findings: play behaviour by Holloway and Suter (2004); social isolation by Boissy et al. (2005); and brushing by Tamioso et al. (2017).

TABLE 12 - MAIN QUESTIONS (Q) AVAILABLE TO 1103 PARTICIPANTS, BEING 388 ORDINARY CITIZENS FROM CURITIBA, PARANA, BRAZIL (OB), 350 ORDINARY CITIZENS FROM CLERMONT-FERRAND, THEIX, FRANCE (OF), 248 VETERINARIANS (VB), 92 BIOLOGISTS (BB) AND 25 ANIMAL SCIENTISTS (AB) FROM CURITIBA, PARANA, BRAZIL; NOVEMBER 2014 TO MAY 2016

| Questions | Content | Options of answers |
|---|---|---|
| Q01 | Have you ever heard of animal welfare? | Yes, I know what animal welfare is; Yes, I know the subject superficially; No, I have never heard of animal welfare. |
| Q02 | If yes, what do you think animal welfare consists of? | Open question. |
| Q03 | Do you think welfare is taken into consideration for farm animals? | Yes, fully; Yes, most of the times; Yes, half of the times; Yes, a few times; No, never; I do not know. |
| Q04 | What are the most important aspects of animal farming that contribute to good animal welfare? | Open question. |
| Q05 | How often do you have contact with sheep? | Almost every day; 1-3 times a week; 1-3 times a month; A few times a year; Never. |
| Q06 | In a scale from 1 to 5, please select the rating that best describes your opinion:<br>Sheep that are healthy and grow well have their welfare guaranteed.<br>Sheep that are raised indoors, under intensive management systems, have low levels of welfare.<br>Sheep are capable of feeling emotions, such as fear and happiness, in addition to suffering.<br>Sheep clearly express how they feel, that is why it is easy to identify if they are in positive or negative situations. | 1 strongly disagree; 2 disagree; 3 neutral/unsure; 4 agree; 5 strongly agree. |
| Q07 | In a scale from 1 to 5, classify the management procedures that are frequently performed on sheep farms according to your perception of sheep suffering: identification, castration1, tail docking, shearing, reproductive techniques and weaning. | 1; 2 ; 3; 4; 5; I do not know<br>1 no suffering; 2 mild suffering; 3 moderate suffering; 4 severe suffering; 5 very severe suffering. |
| Q08 | The same management procedures from the previous question are described below, with definitions on how they are commonly performed. Rate them again according to your perception of sheep suffering:<br>Identification: through ear notching | 1; 2 ; 3; 4; 5; I do not know<br>1 no suffering; 2 mild suffering; 3 moderate suffering; 4 severe suffering; 5 very severe suffering. |

| | | |
|---|---|---|
| | or punching, tattooing, ear tagging or micro-chipping.<br>Castration: removal or destruction of the testicles, through rubber rings, emasculator/burdizzo or surgery.<br>Tail docking/ tail removal: through rubber rings, cauterization using a hot docking iron or surgery.<br>Shearing: cutting or shaving the fleece/wool, though the use of electric shears, shearing machines or scissors.<br>Reproductive techniques: artificial insemination, synchronization of estrus (through the use of intravaginal sponge impregnated with progestagen) and laparoscopic embryo transfer.<br>Weaning: separation of ewes and lambs before the lambs reach 6 months of age. | |
| Q09 | In a scale from 1 to 5, classify the ability of each animal to feel emotions: pigeon, butterfly, human baby, rat, dog, chicken, fish, sheep, cattle, cockroach and wolf. | 1; 2 ; 3; 4; 5; I do not know<br>1 the animal does not feel emotions; 5 the animal certainly feels emotions; intermediate values are equivalent to a growing capacity to feel emotions. |
| Q10-12 | Watch the video below and describe in 3 adjectives, at most, how the animal is feeling. | Open questions. |
| Q13-15 | Watch the video again and choose, at most, 3 adjectives that best describe how the animal is feeling. | Relaxed; Curious; Nervous; Confident; Distressed; Content; Scared; Anxious; Fearful; Agitated; I do not know; It is not possible to know how the animal feels; Sheep do not feel.<br>In Portuguese (for OB, VB, BB and AB): Calmo; Curioso; Nervoso; Dominante; Estressado; Alegre; Assustado; Ansioso; Com medo; Agitado; Eu não sei; Não é possível avaliar como o animal sente; Ovinos não sentem.<br>In French (for OF): Calme; Curieux; Nerveux; Confiant; Stressé; Joyeux; Effrayé; Anxieux; Peureux; Agité; Je ne sais pas ; Impossible d'évaluer ce que l'animal ressent ; Les moutons ne ressentent pas. |

Responses to the open questions 02 and 04 were classified according to the Five Freedoms (Farm Animal Welfare Council 2009). Responses that could not be classified into the Five Freedoms were considered as “other”. We categorized the responses for Q11, Q12 and Q13 by the valence of the adjectives cited for each video, as 1) Positive, 2) Negative and 3) Others (e.g. “I do not know”, “I could not open the video”, “I do not want to watch the video” and adjectives that could not be categorized as positive or negative, such as “adapted”).

Data were analyzed by comparing responses of OB and OF, as well as OB, VB, BB and AB. Gender, age and education were considered in the comparisons within groups. For comparisons within VB, BB and AB, only gender and age were considered, since all veterinarians, biologists and animal scientists were, at least, graduated. Comparisons between cities and gender were performed using the Mann-Whitney test; the Kruskal-Wallis test, followed by Dunn's post hoc test, was used for comparisons among Brazilian participants, and when the variables age and education were considered. Such tests were applied for Q01, Q03, Q05, Q06, Q07, Q08 and Q09. The Wilcoxon test for pair-wise comparisons was used between Q07 and Q08. All tests were applied using Minitab software, version 17.

## 6.3 RESULTS AND DISCUSSION

### 6.3.1 Demographic data

The demographic data presented on Table 2 show that, in general, most respondents were females, aged 18-29 years-old and had higher education (complete or post-graduation) (TABLE 13). A higher number of female participants in our survey is in accordance with gender distribution in Curitiba, Parana, Brazil (47.7% males and 53.3% females) (IBGE, 2010) and Clermont-Ferrand, France (48.0% males and 52.0% females ) (INSEE, 2014), and it may be related to the fact that women have greater concern and empathy toward animal welfare and sentience (KNIGHT et al., 2004; HERZOG, 2007); consequently, they are probably more motivated to participate in this type of study. A higher number of younger participants and respondents with higher education were also expected, as they seem to show higher interest by animal welfare issues (KELLERT; BERRY, 1980, 1987); however, this may be also related to their potential closer stance regarding internet use. High

participation of younger respondents is in accordance with age distribution in Curitiba, Parana, Brazil (26.4% aged 15-19 years-old) (IBGE, 2010), but not in Clermont-Ferrand, France (38.1% aged 50 years-old or more) (INSEE, 2014).

TABLE 13 - DEMOGRAPHIC DATA OF 1103 RESPONDENTS TO A SURVEY ON ANIMAL WELFARE AND SENTIENCE, NOVEMBER 2014 TO MAY 2016

| Variable | Categories | Number of respondents (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Ordinary citizens, Brazil / Population from Curitiba (OB) | Ordinary citizens, France / Population from Clermont-Ferrand (OF) | Veterinarians, Brazil (VB) | Biologists, Brazil (BB) | Animal Scientists, Brazil (AB) | Total |
| Gender | Male | 114 (29.4) | 136 (38.9) | 65 (26.2) | 22 (23.9) | 7 (28.0) | 344 |
| | Female | 274 (70.6) | 214 (61.1) | 183 (73.8) | 70 (76.1) | 18 (72.0) | 759 |
| Age | 18-29 | 192 (49.5) | 92 (26.3) | 96 (38.7) | 52 (56.5) | 19 (76.0) | 451 |
| | 30-39 | 94 (24.2) | 85 (24.3) | 90 (36.3) | 22 (23.9) | 4 (16.0) | 295 |
| | 40-49 | 47 (12.1) | 68 (19.4) | 32 (12.9) | 8 (8.7) | 2 (8.0) | 157 |
| | 50 or more | 55 (14.2) | 105 (30.0) | 30 (12.1) | 10 (10.9) | 0 (0.0) | 200 |
| Education | Secondary or less | 37 (9.5) | 68 (19.4) | - | - | - | 105 |
| | Higher (in progress or interrupted) | 116 (29.9) | 37 (10.6) | - | - | - | 153 |
| | Higher (complete) | 92 (23.7) | 60 (17.1) | 91 (36.7) | 30 (32.6) | 15 (60.0) | 288 |
| | Higher (post-graduation) | 143 (36.9) | 185 (52.9) | 157 (63.3) | 62 (67.4) | 10 (40.0) | 557 |
| Total | | 388 (100) | 350 (100) | 248 (100) | 92 (100) | 25 (100) | 1103 |

### 6.3.2 General animal welfare issues

No significant differences were found between OB and OF for their knowledge about animal welfare ($P>0.05$), as 43.5% OB and 60.3% OF have heard of the subject superficially, and 42.3% OB and 35.1% OF have heard of the subject more deeply. The results indicate that animal welfare might be an important theme for the citizens. A total of 15.2% OB responded that they have never heard of animal welfare, as compared to 0.0% VB, 1.1% BB and 0.0% AB ($P<0.01$). Schnettler et al. (2008) also found that 17% of the consumers in Chile stated that they did not have knowledge about animal welfare. Age differences were noted only among BB respondents. All BB aged 50 years old or more (10; 100%) claimed that they know

about animal welfare, when compared to younger respondents, aged 18-29 years-old (71.2%) ($P<0.05$). This fact may be related to the level of animal welfare teaching in Brazil. In veterinary and animal science areas, animal welfare teaching may still be considered limited (MOLENTO; CALDERON, 2009). There is no animal welfare teaching in the curriculum of Brazilian biologists, suggesting that the issue may be even more incipient; consequently, younger biologists might show little knowledge about the subject due to lack of exposure to animal welfare issues during their graduate degree studies. Younger biologists have also less professional experience compared to older biologists, who may have had more opportunity to face animal welfare issues. Significant differences concerning education were observed for OB. Most OB with secondary or less education (29.7%) reported that they have never heard of animal welfare, differing from other respondents ($P<0.01$). Such result indicates a positive correlation between education and knowledge about animal welfare, in agreement with other studies showing positive association between education and animal welfare perception and behaviour (KELLERT; BERRY, 1980; MARÍA, 2006).

Terms related to the freedom from fear and distress were the most used to define animal welfare, cited 27.0% of the times by OB, 33.4% by OF, 24.8% by VB, 25.9% by BB and 21.9% by AB (FIGURE 13). Te Velde, Aarts and van Woerkum (2002) also reported that the respondents defined animal welfare mostly in terms of physical and mental well-being. The results point to an association of the definition of animal welfare with emotional states by the respondents.

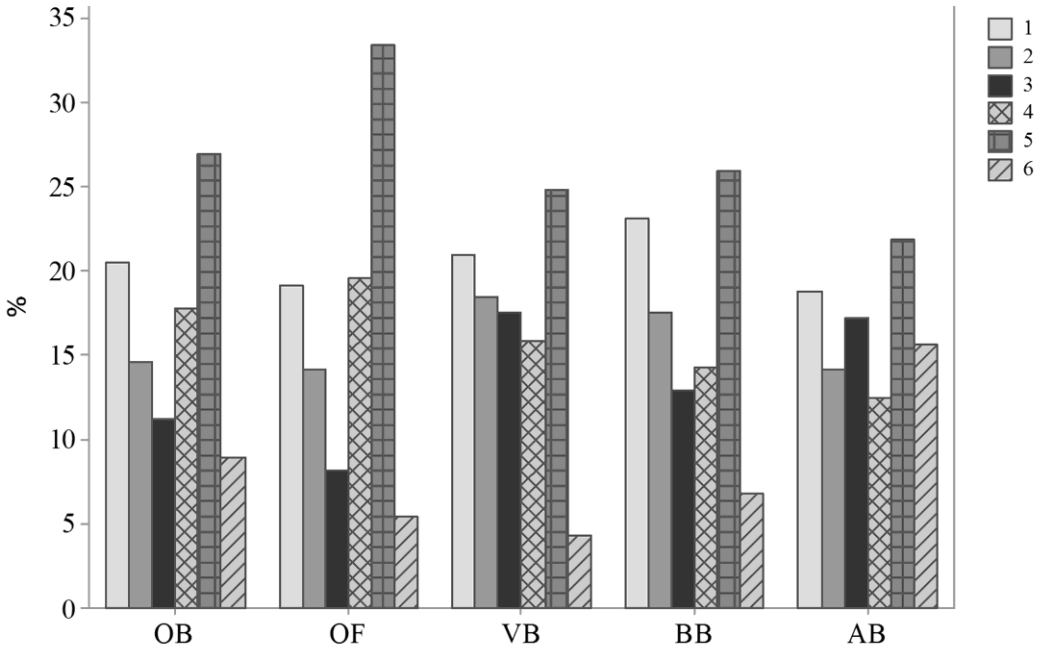


FIGURE 13 - DEFINITION OF ANIMAL WELFARE (Q02), CONSIDERING 1 FREEDOM FROM HUNGER, THIRST AND MALNUTRITION 2 FREEDOM FROM PAIN, INJURY AND DISEASE, 3 FREEDOM TO EXPRESS NORMAL BEHAVIOUR, 4 FREEDOM FROM DISCOMFORT, 5 FREEDOM FROM FEAR AND DISTRESS AND 6 OTHERS, ACCORDING TO 1103 RESPONDENTS, BEING 388 ORDINARY CITIZENS FROM CURITIBA, PARANA, BRAZIL (OB), 350 ORDINARY CITIZENS FROM CLERMONT-FERRAND, THEIX, FRANCE (OF), 248 VETERINARIANS (VB), 92 BIOLOGISTS (BB) AND 25 ANIMAL SCIENTISTS (AB) FROM CURITIBA, PARANA, BRAZIL; NOVEMBER 2014 TO MAY 2016

A total of 46.9% OB and 3.7% OF believed that welfare is not taken into consideration for farm animals ($P<0.01$) (FIGURE 14). Such difference is likely multifactorial, potentially due to different animal welfare scenarios and to different perceptions in both cities. European countries dispose of a great availability of labelled welfare-friendly products (VEISSIER et al., 2008), higher than in Brazil (REMONATO; SOUZA; MOLENTO, 2017, in press); consequently, the French consumer may have the idea that farm animals experience varied levels of welfare, in addition to the fact that the consumers have more options and more information on the products they buy. In studies by Evans and Miele (2008) and Miele and Evans (2010), French participants tended to associate quality products (as “Label Rouge”) and local, regional products with higher animal welfare. However, a recent research revealed that specific welfare aspects assessed in industrial broiler farms were superior in South Brazilian flocks than in Belgian flocks (TUYTTENS et al., 2015). In addition, comparing broiler chicken welfare in certified and non-certified intensive

farms in South of Brazil, Souza et al. (2015) found no differences for some broiler chicken critical welfare issues, such as lameness, panting and contact dermatitis, pointing to the need for the development of more rigorous standards in certification schemes. Significant differences were also found among Brazilian respondents; OB and BB (29.3%) believed that welfare is not taken into consideration for farm animals, in comparison with VB (18.5%) and AB (12.0%) ($P<0.01$) (FIGURE 14). Te Velde, Aarts and van Woerkum (2002) observed that consumers showed a negative perception of the life of farm animals, citing environmental aspects, as lack of space, fresh air and light, and emphasized values related to freedom to move and freedom to fulfill natural desires. Higher perception of consideration of animal welfare by VB and AB demands further studies. We hypothesize that it may be related to the desensitization of these professionals regarding animal welfare issues throughout academic years (PAUL; PODBERSCEK, 2000). However, it may also be related to a more detailed knowledge of animal production scenarios by VB and AB. It is a complex discussion since it involves knowledge regarding the actual level of consideration of farm animal welfare issues. It is also very relevant for animal welfare, due to the impact these professionals have in many different decisions related to animal farming. Significant gender differences were noted among VB: 22.4% females believed that the welfare of farm animals is not considered, in comparison with 7.7% males ($P<0.05$), which suggests higher perception of welfare issues by women, in agreement with other studies (TAYLOR; SIGNAL, 2005). These results are also in agreement with those of Paul and Podberscek (2000); the authors observed that female students reported similar levels of empathy to animals throughout graduate studies, as opposed to male students, who showed less empathy each successive year.

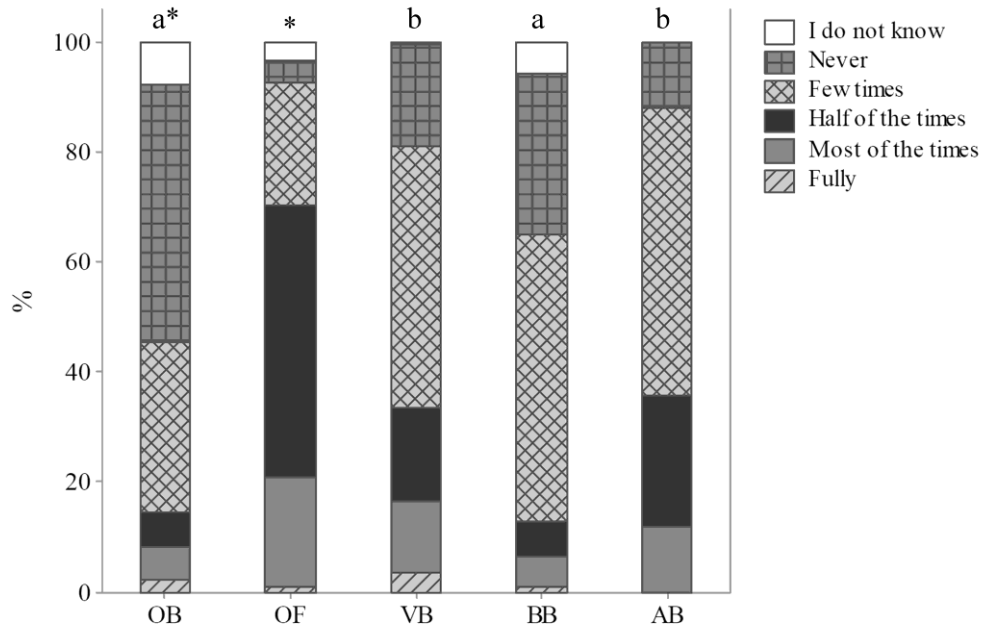


FIGURE 14 - CONSIDERATION OF WELFARE IN THE ANIMAL FARMING SCENARIO (Q03), ACCORDING TO 1103 RESPONDENTS, BEING 388 ORDINARY CITIZENS FROM CURITIBA, PARANA, BRAZIL (OB), 350 ORDINARY CITIZENS FROM CLERMONT-FERRAND, THEIX, FRANCE (OF), 248 VETERINARIANS (VB), 92 BIOLOGISTS (BB) AND 25 ANIMAL SCIENTISTS (AB) FROM CURITIBA, PARANA, BRAZIL; NOVEMBER 2014 TO MAY 2016; THE ASTERISK INDICATES SIGNIFICANT DIFFERENCES BETWEEN OB AND OF ($P<0.05$, MANN-WHITNEY TEST); LETTERS INDICATE SIGNIFICANT DIFFERENCES BETWEEN RESPONDENTS IN CURITIBA, PARANA, BRAZIL ($P<0.05$, KRUSKAL-WALLIS TEST)

Aspects related to freedom from discomfort were cited 31.3%, 36.8%, 27.7% and 34.0% of the times by OB, OF, VB and BB, respectively, as the most important issues of animal farming that contribute to good animal welfare. For AB, aspects related to freedom from hunger, thirst and malnutrition contribute the most to good animal welfare, mentioned 25.7% of the times. Aspects related to animal nutrition (feed and water), animal health, in addition to environmental aspects were also acknowledged by Belgian citizens and farmers in a study by Vanhonacker et al. (2008). Our results are in agreement with these findings, suggesting higher societal concern about comfort and nutritional aspects of animal welfare. Such results also add information on the issues the studied participants believed to be important for farm animal welfare, as for the definition of animal welfare, the majority acknowledged aspects related to freedom from fear and distress.

### 6.3.3 Proximity to sheep, and sheep welfare and sentience

Ordinary citizens from Curitiba and OF did not differ on their responses about their contact with sheep (P>0.05). Among Brazilian respondents, 48.7% OB and 50.0% BB responded to have no contact with sheep, in comparison with 23.8% VB and 12.0% AB (P<0.01), an expected result related to a more frequent interaction of veterinarians and animals scientists with farm animals. In general, the majority of respondents did not have contact with sheep, which is in accordance with literature findings showing that, in a modern society, humans spend little time in physical contact with animals (MALLER et al., 2006).

When asked if sheep that are healthy and grow well have their welfare guaranteed, 21.6% OB and 32.9% OF agreed (P<0.01) (FIGURE 15 - I). The result points to a higher perception of association between animal welfare and physical conditions by French respondents. Among respondents in Curitiba; 15.5% OB and 11.3% VB strongly agreed with the statement, in comparison with 6.5% BB and 4.0% AB (P<0.05); BB and AB differed between them and from OB and VB (P<0.05) (FIGURE 15 - I). It was expected that professionals that interact with farm animals, mainly veterinarians and animal scientists, would have a similar perception. In a survey with students of a veterinary faculty, 40% agreed that if animals are producing (e.g., gaining weight or producing eggs) it means that they have good welfare (HELESKI; MERTIG; ZANELLA, 2005). The results point to similar perceptions of OB and VB about the association between animal welfare and production; more research is necessary to investigate why veterinarians, animal scientists and biologists, mainly the first two, showed different perceptions of such subject. Significant age differences were found among OB and OF for such statement; 34.0% OB aged 40-49 years-old agreed that sheep that are healthy and grow well have their welfare guaranteed, higher than other age classes (P<0.01). A similar result was found for OF; most respondents aged 40-49 (20.6%) and 50 years-old or more (19.05%) strongly agreed with such statement (P<0.01). The results suggest that older ordinary citizens tend to view animal welfare mainly in terms of physical health. Respondents from OF also differed on their perception depending on educational level. Most OF with secondary or less educational level (45.6%) agreed with the statement, differing from other groups (P<0.01), indicating that participants with lower education might associate quality of life in farm animals with physical conditions.

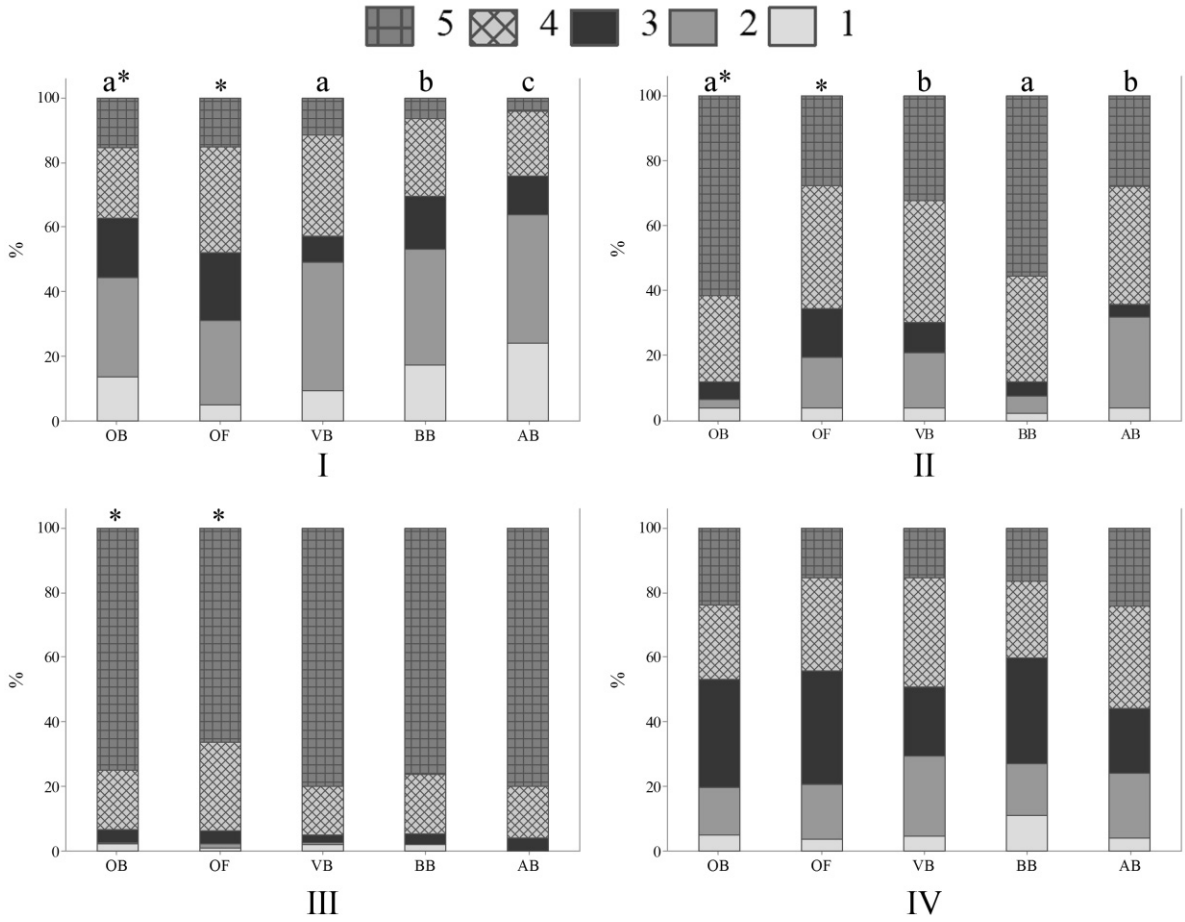


FIGURE 15 - LEVELS OF AGREEMENT CONCERNING SHEEP WELFARE AND SENTIENCE (Q06), BY 1103 RESPONDENTS, BEING 388 ORDINARY CITIZENS FROM CURITIBA, PARANA, BRAZIL (OB), 350 ORDINARY CITIZENS FROM CLERMONT-FERRAND, THEIX, FRANCE (OF), 248 VETERINARIANS (VB), 92 BIOLOGISTS (BB) AND 25 ANIMAL SCIENTISTS (AB) FROM CURITIBA, PARANA, BRAZIL; NOVEMBER 2014 TO MAY 2016. (I) SHEEP THAT ARE HEALTHY AND GROW WELL HAVE THEIR WELFARE GUARANTEED; (II) SHEEP THAT ARE RAISED INDOORS, UNDER INTENSIVE MANAGEMENT SYSTEMS, HAVE LOW LEVELS OF WELFARE; (III) SHEEP ARE CAPABLE OF FEELING EMOTIONS, SUCH AS FEAR AND HAPPINESS, IN ADDITION TO SUFFERING; (IV) SHEEP CLEARLY EXPRESS HOW THEY FEEL, THAT IS WHY IT IS EASY TO IDENTIFY IF THEY ARE IN POSITIVE OR NEGATIVE SITUATIONS; 1 = STRONGLY DISAGREE; 2 = DISAGREE; 3 = NEUTRAL/UNSURE; 4 = AGREE; 5 = STRONGLY AGREE; THE ASTERISK INDICATES SIGNIFICANT DIFFERENCES BETWEEN OB AND OF ($P < 0.05$, MANN-WHITNEY TEST); LETTERS INDICATE SIGNIFICANT DIFFERENCES BETWEEN RESPONDENTS IN CURITIBA, PARANA, BRAZIL ($P<0.05$, KRUSKAL-WALLIS TEST).

Regarding “sheep that are raised indoors, under intensive management systems, have low levels of welfare”, 61.3% OB and 38.0% OF strongly agreed with the statement ($P<0.01$) (FIGURE 15 - II). The results show higher perception of association between outdoor systems and higher levels of welfare by OB. A total of 2.8% OB and 5.4% BB disagreed with such statement, when compared with 16.9%

VB and 28.0% AB (P<0.01) (FIGURE 15 - II). The results present a higher perception of animal welfare in terms of outdoor access by OB and BB, in contrast to VB and AB. The fact that VB and AB in our study showed lower perception of animal welfare in outdoor systems than OB and BB might be due to greater knowledge by veterinarians and animal scientists concerning the production systems. Extensive farming provides the animals the opportunity to engage in natural behaviour; however, it exposes them to more environmental challenges. Confinement systems protect the animals from predation, some parasites and harsh weather. Such factors must be balanced, and they were probably taken into consideration by VB and AB on their response to this statement. Significant differences among education groups were found for OF. Most OF having secondary or less educational level (39.7%) strongly agreed that sheep that are raised indoors have low levels of welfare, when compared with other groups (P<0.05), showing that OF with lower education relate animal welfare to outdoor access.

A total of 75.0% OB strongly agreed that “sheep are capable of feeling emotions, such as fear and happiness, in addition to suffering”, in comparison with 66.3% OF (P<0.05) (FIGURE 15 - III). The fact that less participants in France strongly agreed that sheep are capable of feeling emotions is an interesting result, as in Clermont-Ferrand there is a high quantity of sheep producers, so consequently, we expected the French participants to be more familiar to sheep and, then, attribute more emotional capacities to them, as reported by Morris, Knight and Lesley (2012). However, lower attribution of emotions to animals by French respondents was noted before (EVANS; MIELE, 2008). Evans and Miele (2008) found that certain French participants believed that some of the proposed measures of Welfare Quality®, including positive emotional states, are more suited for human than for animal welfare. No significant differences were found among OB, VB, BB and AB for the statement (P>0.05); in general, the majority of respondents agreed or strongly agreed that sheep experience emotions (FIGURE 15 - III). The results corroborate findings by Rasmussen, Rajecki and Craft (1993) and Morris, Knight and Lesley (2012), in which the majority of respondents believed that animals experience emotions. When gender was considered, significant differences were found for OF and VB. Female OF showed higher perception of sheep emotions, as 70.6% females strongly agreed that sheep feel emotions, in comparison with 59.5% males (P<0.05), in agreement with previous results in our study that showed higher levels of

perception toward animal welfare and sentience by women. A total of 83.1% female VB strongly agreed that sheep are capable of feeling emotions, such as fear and happiness, in addition to suffering, in contrast with 70.8% male VB ($P<0.05$). Phillips and McCulloch (2005) also reported that female students were more opposed to animal suffering than male students. Educational level differences were also noted among BB and AB. A total of 37.5% BB aged 40-49 years-old strongly agreed with the statement, when compared with 78.8% BB aged 18-28 years old ($P<0.01$); 50.0% AB aged 40-49 years-old strongly agreed with the statement, in comparison with 84.2% AB aged 18-29 years old and 100% aged 30-39 years-old ($P<0.01$). The results suggest higher perception of sheep sentience by younger biologists and younger and middle-aged animal scientists.

When asked if sheep clearly express how they feel, that is why it is easy to identify if they are in positive or negative situations, differences among groups were not observed, with an overall agreement of 66.2% ($P>0.05$) (FIGURE 15 - IV). However, significant age differences were found for VB and OF. Most VB aged 40-49 (46.9%) and 50 years-old or more (40.0%) agreed with the statement, in comparison with younger participants, aged 18-29 (31.3%) and 30-39 years-old (30.0%) ($P<0.01$). A total of 36.8% OF aged 40-49 years-old agreed with the statement, differing from other age groups ($P<0.05$). The findings suggest a higher perception and identification of sheep emotions by VB and OF aged around 40-49 years-old. Significant differences among educational levels were also noted for OF, as 33.8% OF having secondary or less educational level agreed with such statement, differing from other groups ($P<0.01$). The results point to higher perception of animal sentience by OF with lower educational levels, in potential disagreement with other studies that show no significant association between pro-animal welfare attitudes and educational levels (SIGNAL; TAYLOR, 2006).

### 6.3.4 Sheep suffering

The perception of suffering differed significantly from the first and second questions for the following management procedures amongst OB: identification, castration, tail docking, reproductive techniques and weaning ($P<0.05$) (FIGURE 16); amongst OF: identification, tail docking, reproductive techniques and weaning ($P<0.05$) (FIGURE 16); amongst VB: castration, tail docking and reproductive

techniques ($P<0.05$) (FIGURE 16); amongst BB: castration, tail docking and reproductive techniques ($P<0.01$) (FIGURE 16) and amongst AB: reproductive techniques ($P<0.01$) (FIGURE 16). Significant differences between the two questions were expected, since some participants, as OB and OF, may not have been used to such procedures and, consequently, might not have knowledge about them. In addition, when the questions were introduced for the second time, the explanations might have elicited higher concern from the participants. All invasive management procedures that are routinely performed in the sheep industry have the potential to cause stress and suffering to sheep, which may last a few to several days. Due to differences in both questions, including for VB, it seems necessary to discuss more about suffering caused by invasive management procedures and also improve veterinary teaching content on these issues in order to increase recognition that sheep are sentience beings.

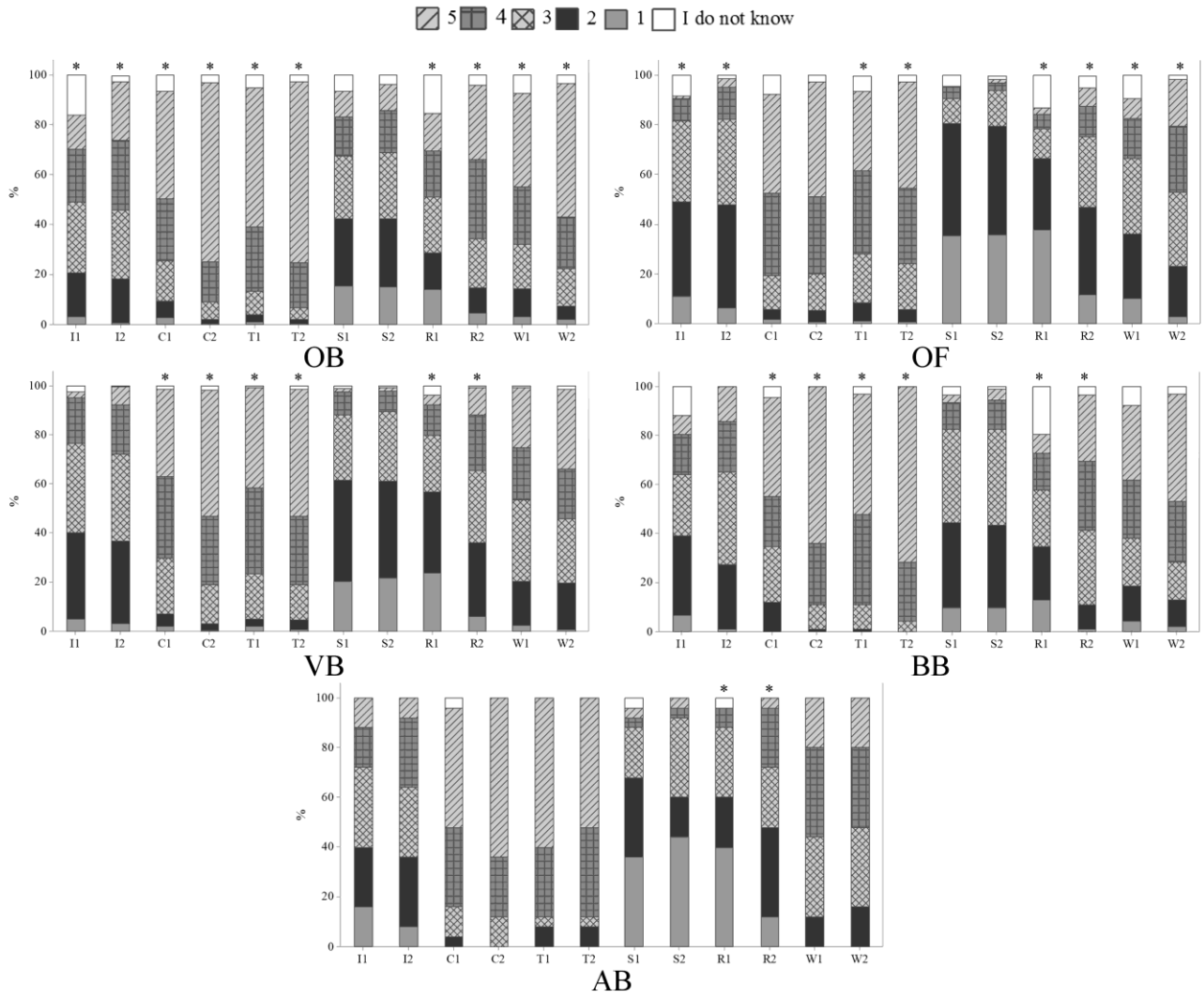


FIGURE 16 - LEVELS OF SUFFERING ATTRIBUTED TO DIFFERENT MANAGEMENT PROCEDURES (Q07 AND Q08) BY 1103 RESPONDENTS, BEING 388 ORDINARY CITIZENS FROM CURITIBA, PARANA, BRAZIL (OB), 350 ORDINARY CITIZENS FROM CLERMONT-FERRAND, THEIX, FRANCE (OF), 248 VETERINARIANS (VB), 92 BIOLOGISTS (BB) AND 25 ANIMAL SCIENTISTS (AB) FROM CURITIBA, PARANA, BRAZIL; NOVEMBER 2014 TO MAY 2016. 1 = NO SUFFERING; 2 = MILD SUFFERING; 3 = MODERATE SUFFERING; 4 = SEVERE SUFFERING; 5 = VERY SEVERE SUFFERING; I1 = IDENTIFICATION1; I2 = IDENTIFICATION2; C1 = CASTRATION1; C2 = CASTRATION2; T1 = TAIL DOCKING1; T2 = TAIL DOCKING2; S1 = SHEARING1; S2 = SHEARING2; R1 = REPRODUCTIVE TECHNIQUES1; R2= REPRODUCTIVE TECHNIQUES2; W1 = WEANING1; W2 = WEANING2; THE ASTERISK INDICATES SIGNIFICANT DIFFERENCES BETWEEN THE FIRST AND SECOND QUESTIONS CONCERNING SHEEP SUFFERING DUE TO MANAGEMENT PROCEDURES (P<0.05, WILCOXON TEST).

Citizens differed on their perception toward almost all the management procedures: a very severe suffering was attributed to sheep by 13.5% OB and 1.1% OF in identification1 and 23.7% OB and 3.4% OF in identification2; 71.8% OB and 46.3% OF in castration2; 55.7% OB and 31.7% OF in taildocking1 and 72.7% OB and 42.9% OF in taildocking2; 10.1% OB and 0.6% OF in shearing1 and 10.3% OB

and 1.1% OF in shearing2; 15.0% OB and 2.3% OF in reproductive techniques1 and 29.7% OB and 7.1% OF in reproductive techniques2; 37.4% OB and 8.0% OF in weaning1 and 53.6% OB and 18.9% OF in weaning2 ($P<0.01$ for all comparisons). These results pointed to higher perception of sheep suffering by OB, which might be related to the fact that French participants believed, more than Brazilian respondents, that animal welfare is taken into consideration in the livestock scenario. Consequently, French citizens might have the idea that the management procedures frequently performed in the sheep industry cause low levels of suffering to the animals.

Significant differences were also found amongst the Brazilian groups. A total of 16.1% OB believed that sheep suffer very severely in identification1, higher perception of suffering than 2.5% VB, 8.6% BB and 12.0% AB ($P<0.01$). The majority OB (74.1%) believed that sheep suffer very severely during castration2, in comparison with 52.5% VB, 64.1% BB and 64.0% AB ($P<0.01$); VB, BB and AB did not differ statistically. The perception of tail docking2 was higher by OB (74.6%) and BB (71.7%) than by VB (52.6%) and AB (52.0%) ($P<0.01$). Similarly, 16.9% OB and 11.2% BB responded that sheep suffer severely, when compared with 9.3% VB and 4.2% AB ($P<0.01$). A total of 17.7% OB and 9.5% BB attributed very severe suffering to sheep in reproductive techniques1, as compared to 4.2% VB and 0.0% AB ($P<0.01$). A total of 55.5% OB and 44.9% BB believed that sheep suffer very severely during weaning2, in comparison with 33.1% VB and 20.0% AB ($P<0.01$). The findings indicate that OB and BB had similar perceptions of sheep suffering, as well as VB and AB. Higher perception of pain in sheep by OB and BB suggests a potential demand for higher level of animal welfare during management procedures, and the need for new strategies to increase sensibility and empathy of VB and AB toward pain. Lower perception of suffering in management procedures by VB and AB might be due to decreased sensitivity in the end of graduation, which might persist during the professional life. Paul and Podberscek (2000) found a negative association between year of study and belief in animal sentience, as veterinary students in their later years of study rated some animals as having lower levels of sentience. An alternative interpretation of our results, that professionals in the field may have a more knowledgeable and correct interpretation of suffering signs in animals, seems rejectable, due to the scientific knowledge about stress and suffering during common farming practices (identification through metal and plastic tags: EDWARDS;

JOHNSTON, 1999; tail docking and castration: MELLOR; STAFFORD, 2000; shearing: SANGER et al., 2011; reproductive techniques: BROOM, 1998; weaning: ORGEUR et al., 1999), and also because of the statistical differences in suffering attributed by veterinarians after reading the descriptions of the procedures. These differences may be related to the fragile teaching of animal welfare and pain in Brazilian veterinary programs (BORGES et al., 2013). Therefore, there is a need to protect and promote sensibility during undergraduate courses, as a way to improve perception of pain by VB and AB, since such professionals are involved in decisions regarding animal management.

#### 6.3.4.1 Gender differences affect the perception of sheep suffering

Females showed higher perception of sheep suffering than males. A total of 46.4% female VB attributed very severe suffering to tail docking1, a higher perception than observed by 25.4% males ($P<0.05$), and for tail docking2, 58.5% female VB believed that sheep suffer very severely, in comparison with 35.5% males ($P<0.01$). A total of 5.1% female VB believed that sheep suffer very severely during reproductive techniques1, in comparison with 1.6% males ($P<0.01$), and 12.1% female BB attributed the highest score of suffering to sheep, than observed for male BB: 0.0% ($P<0.05$). For weaning1, 29.0% female VB attributed very severe suffering to sheep, higher perception than found by 11.1% males ($P<0.01$) and 38.2% female BB gave the highest scores of suffering to sheep, in comparison with 11.8% males ($P<0.01$). For weaning2, 39.6% female VB believed that sheep suffer very severely, versus 14.3% male VB ($P<0.01$). Higher concern from women toward management procedures was expected; women tend to react more emotionally and empathetically to animal suffering (ELDRIDGE; GLUCK, 1996; KIELLAND; SKJERVE; ZANELLA, 2009).

#### 6.3.4.2 Age differences affect the perception of sheep suffering

A general high perception of sheep pain was noted amongst older OF (40-49 years-old), VB (40-49 years-old) and AB (40-49 years-old). Most OF aged 30-39 years-old (44.0%) attributed moderate suffering to sheep during identification1 ($P<0.01$). A total of 53.9% OF aged 18-29 years-old attributed no suffering during

shearing1 (P<0.01). Older VB, aged 40-49 (13.3%) and 50 years-old or more (13.3%), attributed severe level of suffering to sheep for shearing2, in comparison with other groups (P<0.05). All AB aged 40-49 years-old attributed moderate suffering to sheep for castration2, higher perception than other age groups (P<0.05). These results contradict literature reports, in which older participants generally show less concern about animal welfare and suffering (KELLERT; BERRY, 1987). More studies are necessary to understand the effect of age on the perception of suffering by the studied groups, mainly veterinarians and animal scientists, as both professionals are directly involved in animal husbandry.

6.3.4.3 Formal educational levels affect the perception of sheep suffering

The influence of educational levels over sheep pain was noted only amongst OF; OF with higher education attributed severe level of suffering for identification1 (18.5%), when compared with other groups (P < 0.05). In addition, OF having secondary or less education (20.6%) and incomplete graduation (24.3%) attributed moderate suffering to sheep to shearing2, a lower perception than other groups (P < 0.05), indicating that higher levels of education might be associated with more positive perception of animal welfare (KELLERT; BERRY, 1980; TOMA et al., 2012). Further research focusing on French respondents would be helpful to better understand the effect of education on animal suffering.

6.3.5 Sentience in different species of animals

FIGURE 17 shows that mammals were given the highest scores of sentience by the participants, followed by birds, fish and invertebrates. Higher scores attributed to dogs and human baby may be due to familiarity and popularity of dogs as companion animals (PHILLIPS; MCCULLOCH, 2005). The wolf was perceived as a highly sentient being by the surveyed participants (FIGURE 17), probably due to its similarities with dogs. Invertebrates received the lowest scores of emotions (FIGURE 17), in line with other findings (KELLERT, 1993). The results are in agreement with several studies that show that there is a positive association between similarities in animals and humans and attribution of mental and emotional states to animals (HERZOG; GALVIN, 1997; NAKAJIMA; ARIMITSU; LATTAL, 2002).

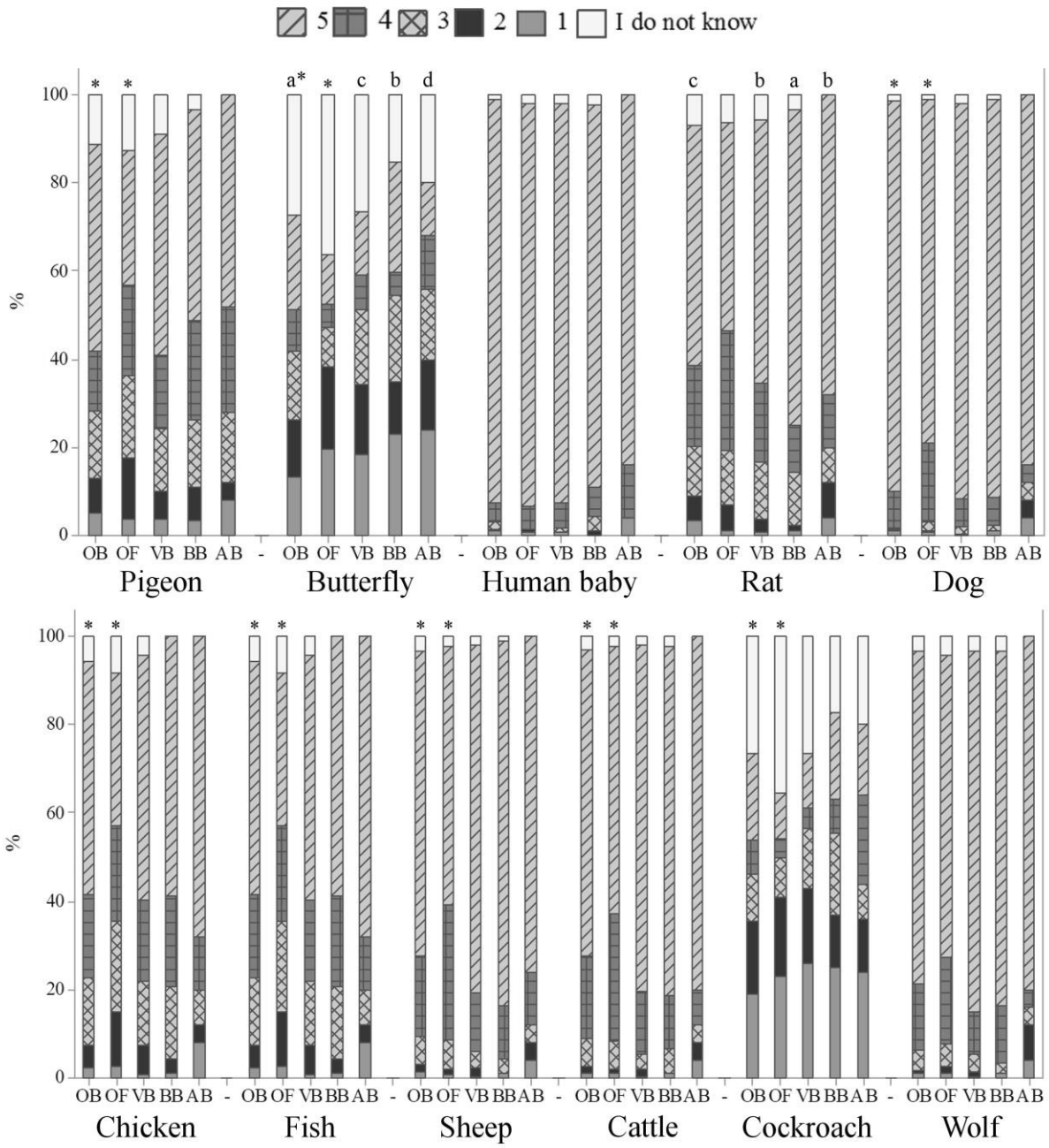


FIGURE 17 - THE ABILITY OF DIFFERENT ANIMALS TO FEEL EMOTIONS (Q09), IN A SCALE FROM 1 TO 5, BEING 1 THE ANIMAL DOES NOT FEEL EMOTIONS, 5 THE ANIMAL CERTAINLY FEELS EMOTIONS AND INTERMEDIATE VALUES ARE EQUIVALENT TO A GROWING CAPACITY TO FEEL EMOTIONS, ACCORDING TO 1103 RESPONDENTS, BEING 388 ORDINARY CITIZENS FROM CURITIBA, PARANA, BRAZIL (OB), 350 ORDINARY CITIZENS FROM CLERMONT-FERRAND, THEIX, FRANCE (OF), 248 VETERINARIANS (VB), 92 BIOLOGISTS (BB) AND 25 ANIMAL SCIENTISTS (AB) FROM CURITIBA, PARANA, BRAZIL; NOVEMBER 2014 TO MAY 2016; THE ASTERISK INDICATES SIGNIFICANT DIFFERENCES BETWEEN OB AND OF ($P < 0.05$, MANN-WHITNEY TEST); LETTERS INDICATE SIGNIFICANT DIFFERENCES BETWEEN RESPONDENTS IN CURITIBA, PARANA, BRAZIL ($P < 0.05$, KRUSKAL-WALLIS TEST).

Significant differences between OB and OF were found for pigeon, butterfly, dog, chicken, fish, sheep, cattle and cockroach (P<0.01) (FIGURE 17); OB attributed higher scores of emotions to such animals (FIGURE 17). For the first time, differences between citizens in Curitiba, Brazil and Clermont-Ferrand, France on the perception of animal emotions are reported, so further studies may contribute to better understand the results. A curious result for the perception of butterfly and rat was found when respondents in Curitiba were compared. A total of 18.4% OB believed that the butterfly does not feel emotions, compared with 24.7% VB, 26.9% BB and 30.0% AB (P<0.05); VB, BB and AB also differed on their perception of emotional capacities in butterflies (FIGURE 17). As butterflies are commonly attributed some aesthetic appeal, compared to other invertebrates, it was expected that they were given higher levels of emotions by all the respondents. Kellert (1993), for example, reported that American respondents disliked and feared many invertebrates, but butterflies were appreciated. On the opposite, 74.2% BB showed the highest perception toward rats, differing from the other groups (P<0.01) (FIGURE 17); VV and AB showed similar perception of emotions in rats (P>0.05) (FIGURE 17). Mice are usually rated the lowest in preference/empathy ranks, due to fear, as they are known to spread diseases (BORGI; CIRULLI, 2015). However, higher perception of sentience in rats by biologists may be due to interactions and familiarity with such animals. Gender differences were observed for all the respondents for some species of animals, except by OB, curiously. Female OF attributed the highest scores to cattle (65.6%), sheep (64.1%) and cockroach (17.7%) than males (57.1%, 53.4% and 13.5%, respectively) (P<0.05). The majority of female VB attributed the highest scores of emotional capacities to butterfly (24.6%) (P<0.01), fish (50.0%) (P<0.05) and cockroach (21.2%) (P<0.05), when compared with male respondents (2.3%, 35.1%, 2.2%, respectively). Among BB respondents, females gave the highest scores of sentience to chicken (62.9%) (P<0.05), fish (47.8%) (P<0.01), sheep (90.0%) (P < 0.01), cattle (87.1%) (P<0.01) and wolf (88.4%) (P<0.05), when compared with males (45.4%, 31.6%, 61.9%, 60.0%, 65.0%, respectively). Female AB differed significantly from males on their perception toward the cockroach, as 26.7% gave the highest score of sentience to such animal, in comparison with 0.0% males (P<0.05). Gender differences regarding the attribution of sentience to animals are expected, as women tend to be more empathetic toward animals. Furnham and

Heyes (1993) also found that females rated the emotional abilities of animals more than males. As noted for the gender differences amongst OF, VB and AB, it is curious that females rated the highest scores of sentience to invertebrates than males. Such result contrasts findings by Bjerke and Østdahl (2014), who reported that females attributed higher preference scores for popular and neutral species more than males, whereas males liked less-preferred animals, as invertebrates. The attribution of preference scores to animals might be related to the degree of empathy the respondents show towards them, and, consequently, attitudes to protect their existence (BJERKE; ØSTDAHL, 2014). However, higher scores of preference might not be associated with sentience recognition and further research is required.

The perception of sentience in some species also differed according to the age groups, except for OF and AB. A total of 40.0% OB aged 40-49 years-old attributed the highest scores of sentience to pigeons, compared with 53.3% participants aged 18-29 years-old ($P<0.05$). A total of 31.7% OB aged 40-49 years-old scored the highest level of emotions to fish, higher than other age groups ($P<0.05$). Concerning the cockroach, 42.4% OB aged 50 years-old or more attributed the highest rate of emotional states to such animal, higher than other age classes ($P<0.05$). The majority of VB aged 19-29 years-old (70.0%) attributed the highest level of sentience to the rat, in comparison with 43.3% aged 40-49 years-old and 53.6% aged 50 years-old or more ($P<0.05$). The perception of VB toward the dog also differed among respondents, as 72.4% respondents aged 50 years-old or more showed the highest scores of emotions, a lower percentage than observed for other groups (18-29: 93.6%; 30-39: 93.3%; 40-49: 96.8%) ($P<0.01$). A total of 90.2% VB aged 18-29 years-old and 86.5% aged 30-39 years old attributed the highest scores of sentience to the wolf, when compared with 77.4% aged 40-49 years-old and 67.9% aged 50 years-old or more ($P<0.05$). The lowest perception toward the butterfly was found by the majority of BB aged 40-49 years old ($P<0.05$). However, the highest perception of chicken was observed by BB aged 40-49 years-old. Concerning the cockroach, the highest level of sentience was attributed by the youngest BB (29.5%) ($P<0.05$). According to the results, it is possible to note a general trend of older ordinary citizens scoring higher affective states to fish and cockroach, differing from groups of professionals who interact with animals, in which younger VB tended to attribute the highest levels of emotions to the rat and wolf and younger BB to the butterfly. In general, there seems to be a negative correlation

between age and interest in animals, as older people seem to show less interest and empathy toward animals (KELLERT, 1996; BJERKE; ØDEGARDSTUEN; KALTENBORN, 1998; PROKOP; TUNNICLIFFE, 2008). However, in our study we found that, in general, older respondents showed higher levels of perception of animal welfare issues (e.g., knowledge about animal welfare, perception and identification of sheep emotions and sheep suffering). Lastly, a significant effect of education was noted among OB for some animals. The majority of OB having secondary or less educational level attributed the highest scores of emotions to pigeon (33.3%), chicken (38.2%) and sheep (51.3%), higher than other groups ($P<0.05$). This is the first study to show the effect of demographic variables on the perception of different groups of respondents from Brazil and France; the results suggest that there is higher perception of emotional capacities for specific animals and amongst specific groups of respondents, indicating that this is a rich area for further research.

### 6.3.6 Videos

FIGURE 18 presents the word clouds with the most cited descriptors for Q10 to Q12. It is possible to note that the most mentioned descriptors in Portuguese and French, respectively, were similar for V1: “feliz”/joyeux” (happy) and “livre”/”libre” (free); V2: “medo”/”peureux” (fearful); V3: “tranquilo” (relaxed) and “bien” (well).


FIGURE 18 - WORD CLOUDS SHOWING THE MOST CITED DESCRIPTORS BY ORDINARY CITIZENS FROM CURITIBA, PARANA, BRAZIL (OB), ORDINARY CITIZENS FROM CLERMONT-FERRAND, THEIX, FRANCE (OF), VETERINARIANS (VB), BIOLOGISTS (BB) AND ANIMAL SCIENTISTS (AB) FROM CURITIBA, PARANÁ, BRAZIL, FOR VIDEOS 1, 2 AND 3, RESPECTIVELY, RESPECTING THE RESPONDENTS' ORIGINAL LANGUAGE. THE WORD CLOUDS CONTAIN ADJECTIVES THAT WERE CITED 3 TIMES AT MINIMUM AND 170 TIMES AT MAXIMUM. LARGER WORDS REPRESENT THE DESCRIPTORS THAT WERE USED MORE FREQUENTLY BY THE RESPONDENTS THAN SMALLER WORDS. 2014-2016. WORKITOUT WORD CLOUDS.

Similar descriptors were found for Q13 to Q15 (TABLE 14). For example, for V1, play behaviour was mainly associated with positive states. Most of respondents attributed the adjectives “content” (“alegre”/”joyeux”) and “curious” (“curioso”/”curieux”) to sheep (TABLE 14). The majority of participants believed that socially isolated sheep in V2 were mainly “scared” (“assustado/”effrayé”), “anxious” (“ansioso”/”anxieux”), “distressed” (“estressado”/”stressé”) and “fearful” (“com medo/”peureux”) (TABLE 14). For V3, most of respondents attributed a “relaxed” (“calmo”/”calme”) and “content” (“alegre”/”joyeux”) state to the sheep being brushed by a familiar observer. The terms used by the respondents from all groups may provide information about which descriptors are more understandable or easy to be applied to practical use in Brazil and France for an array of goals, as for instance the development of Qualitative Behaviour Assessment (WEMELSFELDER et al., 2000) and for improved communication with stock people.

TABLE 14 - ABSOLUTE FREQUENCY (AF) AND PERCENTAGE (%) OF THE MOST CITED DESCRIPTORS BY 388 ORDINARY CITIZENS FROM CURITIBA-PARANA, BRAZIL (OB), 350 ORDINARY CITIZENS FROM CLERMONT-FERRAND, THEIX, FRANCE (OF), 248 VETERINARIANS (VB), 92 BIOLOGISTS (BB) AND 25 ANIMAL SCIENTISTS (AB) FROM CURITIBA, PARANA, BRAZIL, FOR Q14, Q15 AND Q16, CONCERNING VIDEOS 1 (V1), 2 (V2) AND 3 (V3), RESPECTIVELY; NOVEMBER 2014 TO MAY 2016

| Video | Respondents | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | OB | | OF | | VB | | BB | | AB | |
| | Descriptor | AF (%) | Descriptor | AF (%) | Descriptor | AF (%) | Descriptor | AF (%) | Descriptor | AF (%) |
| V1 | Content | 272 (29.6) | Content | 267 (29.7) | Content | 205 (32.5) | Content | 66 (29.1) | Content | 23 (35.9) |
| | Curious | 211 (23.0) | Curious | 236 (26.2) | Curious | 184 (29.2) | Curious | 64 (28.2) | Curious | 19 (29.7) |
| | Agitated | 133 (14.5) | Confident | 145 (16.1) | Relaxed | 129 (20.4) | Agitated | 33 (14.5) | Relaxed | 12 (18.8) |
| V2 | Scared | 257 (23.1) | Anxious | 245 (27.9) | Distressed | 150 (21.3) | Scared | 65 (22.8) | Scared | 16 (22.2) |
| | Fearful | 227 (20.4) | Distressed | 244 (27.8) | Scared | 147 (20.9) | Fearful | 57 (20.0) | Fearful | 14 (19.4) |
| | Distressed | 216 (19.4) | Nervous | 203 (23.1) | Fearful | 132 (18.8) | Distressed | 54 (18.9) | Nervous | 14 (19.4) |
| V3 | Relaxed | 317 (44.3) | Relaxed | 319 (38.2) | Relaxed | 228 (48.5) | Relaxed | 83 (50.9) | Relaxed | 23 (54.8) |
| | Content | 171 (23.9) | Confident | 318 (38.0) | Content | 129 (27.4) | Content | 46 (28.2) | Content | 13 (31.0) |
| | Curious | 68 (9.5) | Content | 138 (16.5) | Curious | 53 (11.3) | Curious | 13 (8.0) | Confident | 3 (7.1) |

For the videos showing positive events (V1 and V3), most OB (68.0% for V1 and 79.6% for V3), OF (66.0% and 90.3%), VB (76.2% and 89.5%), BB (68.5% and 84.8%) and AB (84.0% and 92.0%) attributed adjectives of positive valence to sheep emotions. Concerning the video showing a negative event (V2), 91.5% OB, 89.4% OF, 92.3% VB, 95.6% BB and 92.0% AB believed that sheep were experiencing negative emotions. A higher frequency of correct perceptions by VB, BB and AB was expected. The results show that, in general, the respondents might have understood the valence of sheep emotions; however, this perception needs improvement. There

is a need to reform the teaching provision in animal welfare to refine the recognition of valence of sheep emotions among professionals, so that they can meet societal expectations of higher knowledge regarding animal welfare than ordinary citizens.

Furthermore, the majority of adjectives attributed by the respondents belong to the group of primary emotions, such as fear, anger, anxiety, curiosity, joy and happiness. In our study, very few secondary emotions were attributed to sheep. The low number of secondary emotions given to sheep may be explained by the fact that people do not commonly interact with sheep as companion animals, in comparison with other studies that assessed the attribution of emotions to pets by pet owners. Martens, Enders-Slegers and Walker (2016) found that companion-animal owners attributed basic emotions more commonly than complex emotions to their animals. Alternatively, there may be a belief that animals do not experience secondary emotions, as pride, guilt, embarrassment, shame, although evidences show the contrary (MASSON; MCCARTHY, 1995). This is the first paper to investigate the attribution of emotional states to sheep by different groups of respondents through video recordings, and our results suggest that this is a rich approach that warrants further research.

## 6.4 CONCLUSION

Ordinary citizens in Curitiba and Clermont-Ferrand differed on their perceptions of welfare and sentience both in livestock and more specifically in sheep, and sheep suffering due to management procedures. Citizens from Curitiba showed a higher perception of animal welfare issues. Regarding the Brazilian respondents, ordinary citizens and biologists seemed to have similar perceptions of animal welfare and emotions, higher than those of veterinarians and animal scientists. The latter showed evidences of desensitization as they believed, more than ordinary citizens and biologists, that welfare is taken into consideration for farm animals, and they showed lower perceptions of sheep suffering. Therefore, it seems important to better understand the reasons why they seemed to have lower perceptions of animal welfare issues and to refine animal welfare education presented in their curricula. In addition, the results suggest a link between the perception of animal welfare and sentience to demographic traits, as females and older respondents tended to show higher concerns for animal welfare issues.

## 6.5 ACKNOWLEDGMENTS

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## 7. FINAL CONSIDERATIONS

Scientific assessments of emotional states have an important role on the advancement of animal welfare science. However, little is known on how affective states are regulated, especially because the inference is subjective and, thus, they are difficult to be assessed. On a day-to-day basis, farm animals are constantly exposed to potentially aversive events during handling, separation from group members, environmental and feeding changes, and such conditions may induce chronic stress. There may be real possibilities of exposing animals more frequently to stimuli that generate positive emotions. In this way, it is necessary to develop and improve methods of assessment of emotional states so that the indicators can be evaluated and applied in different maintenance conditions. Behavioral indicators, as ear postures, ear posture changes, eye aperture and tail wagging provided useful information on the positive perception of sheep from different breeds to gentle physical interactions. Results on withers and nasal temperatures, as well as heart rate and some heart rate variability parameters, as RMSSD/SDNN, also added data to the study of positive emotional states in sheep. Furthermore, positive handling to improve animal welfare can be altered by emotional reactivity and social context. Therefore, it is important to consider the relation between social environment and social motivation of the animals.

Studies on the perception of society to animal welfare issues are important as they contribute to the recognition of animal sentience. Farmers tended to associate animal welfare with physical conditions and they showed a positive perception of sheep sentience. Interestingly, years of experience in the sheep industry seemed to have a negative influence over sheep farmers' attitudes to animal welfare. Ordinary citizens from Curitiba, Paraná, Brazil and Clermont-Ferrand, Theix, France presented, in general, different perceptions of animal welfare issues; ordinary citizens from Curitiba seemed to show higher perception of the subject. Ordinary citizens and biologists shared perceptions and seemed to have higher perception of the subject than veterinarians and animal scientists. Gender differences corroborated the fact that women are more empathetic to animal welfare issues. Curiously, older citizens also showed higher perception of studied subjects. The findings suggest that it is important to develop new strategies to increase sensibility and empathy of sheep farmers, as well as veterinarians and animal scientists to animal welfare issues.

Especially for veterinarians and animal scientists, there is a need to promote sensibility during undergraduate courses, based on the scientific knowledge about suffering involved in invasive management procedures.

The results of this thesis contributed to the advancement of research about emotional states in sheep, by studying behavioral and physiological indicators in response to gentle tactile stimulation, which can be taken into account in welfare assessments. Furthermore, our research added new knowledge about the influence of genetic background over sheep responses, which warrants further studies. Data from Brazilian and French respondents may guide specific initiatives to improve perceptions of sheep welfare and sentience. Recognition of sheep suffering may help in developing and endorsing regulations that aim to minimize sheep pain due to invasive management procedures. By showing that animals have rich and complex emotional abilities, the society might recognize and attribute higher value to animal sentience.

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# APPENDIX I – LIST OF MATERIAL PRODUCED FOR PUBLICATION RELATED TO THE THESIS CHAPTERS

| CHAPTER | DISCLOSURE PLACE | TYPE OF MATERIAL | SITUATION | APPENDIX |
|---|---|---|---|---|
| 2 | *Journal of Veterinary Behavior* Journal | Scientific paper | Published, 2017 | II |
| | Proceedings of the Universities Federation for Animal Welfare (UFAW) International Animal Welfare Science Symposium | Abstract | Published and presented as a poster, 2016 | III |
| | Proceedings of the 10th International Veterinary Behaviour Meeting/ 3rd AMVEBBEA International Congress | Abstract | Published and presented as a poster, 2015 | IV |
| 3 | Proceedings of the Universities Federation for Animal Welfare (UFAW) International Animal Welfare Science Symposium | Abstract | Published and presented as a poster, 2017 | V |
| | Proceedings of the 50th Congress of the International Society for Applied Ethology | Abstract | Published and presented as a talk, 2016 | VI |
| 5 | *Ciência Rural* Journal | Scientific paper | Published, 2017 | VII |
| | Proceedings of the *Reunião Anual da Sociedade Brasileira de Zootecnia - SBZ* | Abstract | Published and presented as a poster, 2016 | VIII |
| 6 | Proceedings of the *VII Congresso Brasileiro de Biometeorologia, Ambiência, Comportamento e Bem-Estar Animal* | Extended abstract | Published and presented as a poster and as a talk, 2017 | IX |
| | Proceedings of the *VII Congresso Brasileiro de Biometeorologia, Ambiência, Comportamento e Bem-Estar Animal* | Extended abstract | Published and presented as a poster, 2017 | X |
| | Proceedings of the *Congresso Medvep de Especialidades Veterinárias* | Extended abstract | Published and presented as a poster, 2015 | XI |
| | Proceedings of the *Congresso Medvep de Especialidades Veterinárias* | Extended abstract | Published and presented as a poster, 2015 | XII |

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|---|---|---|---|---|
| 2, 3, 4 | I Simpósio em Bem-estar Animal, Pontifícia Universidade Católica do Paraná - PUCPR | Talk | 2016 | XIII |
| | Proceedings of the 10th International Veterinary Behaviour Meeting/ 3rd AMVEBBEA International Congress | Abstract | Published and presented as a poster, 2015 | XIV |
| | *Clínica Veterinária* Journal | Scientific paper | Published, 2015 | XV |
| 2, 3, 4, 5, 6 | *Laboratório de Bem-estar Animal* (LABEA) Newsletter no. IV | Newsletter | Published, 2014 | XVI |

# APPENDIX II – PAPER

# "BEHAVIOR AND BODY SURFACE TEMPERATURE AS WELFARE INDICATORS IN SELECTED SHEEP REGULARLY BRUSHED BY A FAMILIAR OBSERVER"

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Research

## Behavior and body surface temperature as welfare indicators in selected sheep regularly brushed by a familiar observer

Priscilla Regina Tamioso [a], Daniel Santiago Rucinque [a], Cesar Augusto Taconeli [b], Guilherme Parreira da Silva [b], Carla Forte Maiolino Molento [a,*]

[a] Animal Welfare Laboratory—LABEA, Department of Animal Science, Federal University of Paraná—UFPR, Curitiba, Paraná, Brazil
[b] Department of Statistics, Federal University of Paraná—UFPR, Curitiba, Paraná, Brazil

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ABSTRACT

Research on emotions in farm animals has increasingly contributed to their welfare. We aimed to study behavioral and body temperature responses of dorper and white dorper sheep regularly brushed on their ventral neck, lateral chest, and withers. We performed 3-minute assessments in prebrushing, brushing, and postbrushing phases. Vocalization, ear postures and changes, half-closed eyes, and tail wagging were assessed. We also recorded withers (Tw), anal (Ta), and nasal (Tn) temperatures with an infrared thermometer. Vocalization was infrequent throughout the phases. We identified 3 ear postures frequently performed by the animals: raised up (R), horizontal (H), and backward (B). We noted a longer duration of R rather than H posture before brushing than during brushing. By comparing B and H, we observed that dorper sheep performed the B posture for longer, mainly during than postbrushing. When B and R were compared, sheep expressed the B posture for longer during brushing than both prebrushing and postbrushing phases; dorpers and male sheep also performed the B posture for longer. The results suggest higher performance of B and H postures during the stimulus and that their expression might be associated with a positive, relaxing state of the animals. No significant result was found for ear changes. Sheep showed half-closed eyes mainly in brushing and postbrushing phases, in comparison with the prebrushing phase, indicating that brushing might have elicited a relaxing state in the animals, which might have persisted after the stimulus. Only 4 male sheep wagged their tails, mostly when brushed. We also noted attention-seeking behaviors, including following the observer, leaning against the brush with the head or neck, and stretching the neck when brushed. The results on body temperatures indicated higher variance for Tw in relation to Tn and Ta, which had similar variances. Pairwise comparisons indicated that Tw was higher in postbrushing than in the prebrushing phase; Tn was also higher in the postbrushing phase than in both prebrushing and brushing phases, suggesting an increase in Tw and Tn temperatures after the stimulus. No significant differences were reported for Ta. Brushing might have elicited a positive state in sheep. Ear postures and half-closed eyes may be useful tools for assessing emotions in sheep, as well as withers and nasal temperatures. Furthermore, breed may be a highly significant effect on the expression of ear postures in sheep.



### Introduction

Emotions in animals were described by Charles Darwin in his book "The expression of emotions in man and animals," in 1872 in which the naturalist compared emotional expressions such as gestures, postures, and facial moves associated with specific contexts in human beings and several animal species. Darwin supported the idea that there is continuity between humans and animals regarding their emotional lives. There are many concepts involved in the study of emotions, the majority of which are founded on research involving humans (Kleinginna and Kleinginna, 1981). Emotions may be defined as short and intense affective responses to an event and include physiological, behavioral, cognitive, motivational, motor, and subjective components (Désiré et al., 2006). According to Mendl et al. (2010), the study of emotions in animals considers as important 2 dimensional approaches, valence

* Address for reprint requests and correspondence: Carla Forte Maiolino Molento, Animal Welfare Laboratory—LABEA, Department of Animal Science, Federal University of Paraná—UFPR, Rua dos Funcionários, 1540, Juvevê, 80035-050 Curitiba, Paraná, Brazil. Tel: 55-41-3350-5788.
*E-mail address:* carlamolento@ufpr.br (C.F.M. Molento).

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## APPENDIX III – ABSTRACT

## "SHEEP PERCEIVE BRUSHING AS A POSITIVE STIMULUS: STUDY OF BEHAVIOURAL RESPONSES AND NASAL TEMPERATURE"

PR Tamioso[1], DS Rucinque[1], APO Souza[1], GP Silva[2], CA Taconelli[2], TC Bergstein[3], CFM Molento[1]

[1]Animal Welfare Laboratory - LABEA, Department of Animal Science, Federal University of Paraná - UFPR, Curitiba, Paraná, Brazil.

[2]Department of Statistics, Federal University of Paraná - UFPR, Curitiba, Paraná, Brazil.

[3]Department of *Bioprocess Engineering and Biotechnology* - UFPR, Curitiba, Paraná, Brazil.

*priscillatamioso@gmail.com*

Research on positive affective states in animals has increasingly contributed to their welfare. Events that may elicit positive emotions include play, feeding and, recently, positive judgment towards tactile interactions have been demonstrated in farm animals. We investigated whether sheep perceive brushing as positive, through behavioural and temperature responses. Twenty-seven Dorper and White Dorper sheep were brushed by a familiar observer in three body regions: ventral neck, lateral chest and withers. We performed 3 min focal assessments at pre, during and post-brushing phases. Vocalization, ear changes and postures, presence of half-closed eyes and tail wagging were assessed. We also recorded nasal temperature with an infrared thermometer, twice at each phase. Data were analyzed using descriptive and nonparametric methods, in addition to marginal and linear mixed models. The models considered sex, breed and phase as fixed effects, as well as the random effect of animal for linear mixed models and a correlation structure for marginal models. Vocalization was not frequent throughout the phases. Regarding ear changes, no significant effect was found ($p>0.05$). Sheep changed ear postures 10 (1/42), 6 (0/26) and 7 (0/39) times pre, during and after brushing, respectively. We identified three main ear postures frequently performed: horizontal (H), raised up (R) and backward (B) postures. In relation to the estimated probabilities for the occurrence of ear postures, we observed important breed and phase differences, when comparing B x H ($p<0.05$). During brushing, sheep tended to show a higher proportion of B posture in comparison with H postures. In this case, a frequent performance of B posture may be an indicator of an appeasing state. Comparing R x H postures, we noted a longer duration of R posture pre than during brushing ($p<0.05$). Sheep showed a higher proportion of half-closed eyes during and post-brushing, when both phases were compared to pre-brushing ($p<0.05$). Only four male sheep wagged their tails, mostly during brushing (median: 7.50s; minimum: 4.38s; maximum: 9.03s). Post-hoc pairwise comparisons indicated important differences for mean nasal temperatures pre (33.46±1.87) and post-brushing (34.12±1.58) ($p<0.05$) as well as during (33.25±2.02) and post-brushing ($p<0.05$). No significant differences were noted pre and during the stimulus ($p>0.05$). Our findings suggest that the animals perceived brushing as positive. Ear postures and half-closed eyes have shown to be useful tools for assessing emotional states in sheep. Furthermore, although there is a need for validation, nasal temperature may be a promising measure of emotions in sheep.

## APPENDIX IV – ABSTRACT

## "DO SHEEP PERCEIVE BRUSHING AS POSITIVE? PRELIMINARY DATA"

TAMIOSO, P.R.[1]; GONZALEZ, D.S.R.[1]; BERGSTEIN, T.G.[2]; MOLENTO, C.F.M.[1]
[1]Laboratório de Bem-estar Animal – LABEA, Universidade Federal do Paraná, Curitiba, Paraná, Brasil. priscillatamioso@gmail.com, carlamolento@ufpr.br
[2]Pós-graduação em Engenharia de Bioprocessos e Biotecnologia – PGEBB, Universidade Federal do Paraná, Curitiba, Paraná, Brasil.

Tactile contact may be used to elicit positive emotions in animals. We aimed to study whether brushing was perceived as positive by sheep. Twelve female and nine male Dorper sheep (20±6 months old on average) were brushed in three body regions. We performed 3 min focal observations pre-, during and post-brushing, assessing body movement, vocalization, ear changes and postures, tail wagging and half-closed eyes. We also recorded nasal temperature (Tn), with an infrared thermometer. Behaviour was analyzed by descriptive statistics and temperature data with Friedman test. Sheep moved for 20 (50/0) before, 4 (21/0) during and 15 (56/0) s after brushing, and rarely vocalized. Ear changes were not frequent; the main ear postures exhibited were ears raised up for a median of 59 (146/0) before, ears pointed backward for 97 (180/0) and horizontal ears for 67 (180/0) during and horizontal ears for 73 (180/0) s after brushing. Three male sheep wagged tails during brushing for 7 (9/4.3) s and, from these, one after brushing. Ten and, from these, four animals showed half-closed eyes during and after brushing, respectively, which may be a sign of relaxation. Eighteen animals leant against the brush and stretched the neck while brushed, suggesting elicitation of positive states. The medians for Tn at pre-, during and post-brushing were 33.4, 32.8, 34.6°C, respectively. There were differences ($p<0.05$) between pre- and post-brushing, and during and post-brushing, which may represent potential sensibility of temperature as an indicator of positive emotions. Our results suggest that sheep perceived brushing as positive.

# APPENDIX V – ABSTRACT

# "BEHAVIORAL RESPONSES OF SHEEP SUBMITTED TO HUMAN PRESENCE AND BRUSHING"

PR Tamioso[1], A Boissy[2], X Boivin[2], H Chandèze[2], S Andanson[2], E Delval[2], CA Taconeli[3], GP Silva[3] and CFM Molento[1]

[1]Animal Welfare Laboratory – LABEA, Department of Animal Science, Federal University of Paraná - UFPR, Curitiba, Paraná, Brazil.

[2]UMR1213 Herbivores, *Institut National de la Recherche Agronomique* – INRA, Saint-Genès Champanelle, France.

[3]Department of Statistics, Federal University of Paraná – UFPR, Curitiba, Paraná, Brazil.

*priscillatamioso@gmail.com*

Positive emotional states have been recently studied in farm animals. We investigated the perception of thirty-eight Romane ewes submitted to the presence of a familiar observer (H) and brushing by a familiar observer (B). Sheep belonged to two genetic lines, more (R+) and less (R-) reactive to temporary social separation. Body postures, head orientation, ear changes and postures, eye aperture, tail moves and ingestion were assessed. Data were analyzed using generalized linear models, considering generalized estimating equations and potential intra-animal correlation. The effects of treatment, genetic line and phase (2.5 min pre-, 3.0 min during and 2.5 min post-treatment) were included in the models, in addition to their interactions. Significant treatment and phase interactions were observed for most indicators ($P<0.05$). It was noted that H ewes tended to show less body posture changes in the pre-treatment phase (0.50±0.23) than B ewes (2.06±0.78), whereas during the treatment, the opposite was observed ($P<0.05$). During the treatment, H ewes showed higher number of head orientation changes (14.08±2.32) than B sheep (2.71±1.28) ($P<0.01$), suggesting that B sheep were more relaxed during brushing. In addition, for R+ ewes, H sheep showed more head orientation changes (16.25±2.44) than B sheep (7.07±1.31) ($P<0.01$). During the treatment, a higher number of ear changes was found for the H group ($P<0.01$), and R+ ewes showed higher number of ear changes (10.83±1.06) than R- ewes (7.68±0.87) ($P<0.05$). Higher proportion of raised up or asymmetrical ear posture was noted pre- (0.73±0.05) than during the treatments (0.53±0.06), in which the horizontal ear was performed for longer ($P<0.05$). Among R+ sheep, H sheep showed raised up or asymmetrical ear postures for longer (0.63±0.06) than B sheep (0.45±0.05) ($P<0.05$). It was also found that H ewes had lower proportion of closed or half-closed eyes (0.15±0.04) than B ewes during brushing (0.53±0.06) ($P<0.01$), supporting the fact that brushed sheep experienced a relaxing state. In addition, overall, R+ sheep showed closed or half-closed eyes for longer (0.25±0.04) in comparison with R- sheep (0.13±0.03) ($P<0.01$). Brushed ewes also wagged their tails for longer that non-brushed sheep mainly during (B: 0.16±0.05; H: 0.01±0.003) and after the treatments (0.02±0.009; 0.007±0.002) ($P<0.01$). Among R+ sheep, B ewes spent more time ruminating (0.48±0.08) than H ewes (0.12±0.06) ($P<0.01$). All the behavioral indicators strongly suggest that both treatments induced a relaxing state in sheep, especially during brushing. Comparing more and less reactive sheep provided significant differences which warrant further studies.

# APPENDIX VI – ABSTRACT

## "INDUCING POSITIVE EMOTIONS: CARDIAC REACTIVITY IN SHEEP REGULARLY BRUSHED BY A HUMAN"

Priscilla Regina Tamioso[1], Guilherme Parreira Silva[2], César Augusto Taconelli[2], Hervé Chandèze[3], Stéphane Andanson[3], Carla Forte Maiolino Molento[1], Alain Boissy[3]

[1]Animal Welfare Laboratory – LABEA, Department of Animal Science, Federal University of Paraná - UFPR, Curitiba, Paraná, Brazil.

[2]Department of Statistics, Federal University of Paraná – UFPR, Curitiba, Paraná, Brazil.

[3]UMR1213 Herbivores, *Institut National de la Recherche Agronomique* – INRA, 63122 Saint-Genès Champanelle, France.

Welfare concerns the absence of negative and presence of positive experiences. We assessed cardiac indicators of sheep through a heart rate monitor, as well as ear postures and tail wagging. Thus, 38 female Romane ewes were trained to be brushed by a familiar human (B) on the neck, withers, chest and belly, or exposed to human presence (H). The ewes belonged to two lines: more (R+) or less (R-) reactive to social isolation. Heart rate (HR) and heart rate variability (RMSSD, RMSSD/SDNN and LF/HF ratios) were analyzed using linear models. The models considered treatment, genetic line and phase (pre- (2.5 min), during (3.0 min) and post-exposure (2.5 min)) as fixed effects, including their interactions. The HR during and after brushing was lower than before brushing ($P<0.01$). No differences in RMSSD were found, but the RMSSD/SDNN ratio during the exposure was higher than before or after ($P<0.05$). The RMSSD/SDNN ratio in R- ewes was higher than in R+ ewes ($P<0.01$), revealing a stronger activation of the parasympathetic system in R- sheep. In R+ line, the B ewes had a higher HR than the H ewes ($P<0.01$) whereas in R- line the difference was reversed ($P<0.01$). In R+ line, the LF/HF ratio of the B ewes was lower than in the H ewes ($P<0.01$). Preliminary results on ear postures also indicate a positive perception of brushing, as sheep showed a higher duration of horizontal ears, equal to 105.82 (0/178.63)s, and wagged their tails for 28.83 (0/151.60)s when brushed. The H ewes performed raised up ears for longer, equal to 60.22 (11.12/171.32)s, and wagged their tails for 1.14 (0/8.68)s. Such behavioural variables will be further analyzed. There is a need to better investigate the differences between R+ and R- before concluding that the emotional reactivity can modulate the autonomic responses to positive events.

Keywords: autonomic responses; emotional reactivity; positive indicators; tactile contact.

# APPENDIX VII – PAPER
# "ATTITUDES OF SOUTH BRAZILIAN SHEEP FARMERS TO ANIMAL WELFARE AND SENTIENCE"

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ANIMAL PRODUCTION

## Attitudes of South Brazilian sheep farmers to animal welfare and sentience

**Priscilla Regina Tamioso[1*] Paulo Ricardo Bittencourt Guimarães[2] Carla Forte Maiolino Molento[1]**

[1]Laboratório de Bem-estar Animal (LABEA), Departamento de Zootecnia, Universidade Federal do Paraná (UFPR), 80035-050, Curitiba, PR, Brasil. E-mail: priscillatamioso@gmail.com. *Corresponding author.
[2]Departamento de Estatística, Universidade Federal do Paraná (UFPR), Curitiba, PR, Brasil.

**ABSTRACT**: *We investigated self-reported attitudes of 148 South Brazilian sheep farmers to animal welfare and sentience. Many farmers (73.0%) knew animal welfare superficially. Farmers that worked for longer in the sheep industry and that raised sheep for commercial purposes mentioned more commonly that they had knowledge about animal welfare (P<0.05). Terms related to freedom from hunger, thirst and malnutrition were the most used to define animal welfare, cited 24.9% of the times. The majority claimed that their animals experience good levels of welfare (93.2%), especially farmers that kept bigger flocks (P<0.05). However, many respondents believed that sheep welfare could be improved on their farms (71.6%), mainly farmers with less experience in the sheep industry (P<0.01). High scores of sentience were attributed to sheep by farmers with frequent contact with their animals (P<0.05). According to the farmers, castration causes the highest levels of suffering to sheep (32.4%) and shearing, the lowest (50.0%). South Brazilian farmer knowledge about animal welfare, attitudes to sentience and recognition of suffering need improvement.*
**Key words**: *animal welfare, emotions, opinion, perception.*

## Atitudes de ovinocultores do sul do Brasil em relação a bem-estar e senciência animal

**RESUMO**: *Objetivou-se estudar as atitudes de 148 ovinocultores do sul do Brasil em relação a bem-estar e senciência animal. A maioria dos produtores (73,0%) conhecia bem-estar animal superficialmente. Produtores que trabalhavam mais tempo na indústria ovina e que criavam ovinos para fins comerciais mencionaram mais comumente que tinham conhecimento sobre bem-estar animal (P<0,05). Termos relacionados à liberdade de fome, sede e desnutrição foram os mais usados para definir bem-estar animal, citado 24,9% das vezes pelos produtores. A maioria afirmou que seus animais possuem níveis adequados de bem-estar (93,2%), especialmente produtores que mantinham rebanhos maiores (P<0,05). No entanto, muitos respondentes acreditavam que o bem-estar dos ovinos poderia ser melhorado em suas fazendas (71,6%), principalmente produtores com menos experiência na indústria ovina (P<0,01). Altos escores de senciência foram atribuídos a ovinos por produtores com contato frequente com seus animais (P<0,05). De acordo com os produtores, a castração causa os maiores níveis de sofrimento aos ovinos (32,4%) e a tosquia, os menores (50,0%). O conhecimento de produtores do sul do Brasil sobre bem-estar animal, as atitudes em relação à senciência e o reconhecimento do sofrimento precisam ser melhorados.*
**Palavras-chave**: *bem-estar animal, emoções, opinião, percepção.*

## INTRODUCTION

Investigations about the perception and attitude of farmers to animal welfare have been reported in the literature and revealed important views about the subject. TE VELDE et al. (2002) observed that farmers showed some knowledge about policies and regulation in animal welfare, although they were not prone to alternative ways of farming with special attention to animal welfare. Farmers' perceptions and attitudes may also be directly and strongly associated with their behaviour towards animals and subsequent behaviour of the animals and their production (HEMSWORTH et al., 2002).

Attribution of sentience to animals may also affect human-animal relationships and attitudes toward animals. A positive relation between the recognition of an animal mind, i.e., the extent to which animals have awareness, thoughts and emotions, and animal welfare has been reported (MORRIS et al., 2012). HILLS (1993) noted that Australian farmers supported human dominance over animals and

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## APPENDIX VIII – ABSTRACT

## "PERCEPTION OF SHEEP FARMERS ON SHEEP WELFARE AND SENTIENCE: PRELIMINARY RESULTS"

Priscilla R. Tamioso*[1], Vanessa Souza Soriano[1], Carla Forte Maiolino Molento[1]
*Ph.D. student, Post-Graduation Program in Veterinary Sciences, Federal University of Paraná - UFPR; 1540 Funcionários Street; Curitiba, Paraná, Brazil; [1]Animal Welfare Laboratory, UFPR
*priscillatamioso@gmail.com

Studies on animal sentience contribute to a growing interest in ethical issues and animal welfare. We aimed to study the perception of sheep farmers regarding sheep welfare and sentience. Thus, 148 sheep farmers from different municipalities in Paraná participated in a telephone interview. The questionnaire contained 26 questions on sheep farming, welfare and sentience. The results were analyzed using descriptive statistics. Most of the respondents were male farmers (84.5%) (125/148). Regarding animal welfare, 73.0% (108/148) have heard of the subject, but superficially. When asked about the aspects that contribute to sheep welfare, 48.0% (71/148) considered nutrition, i.e. freedom of hunger and thirst. Most of the farmers claimed that their animals have good welfare (93.2%) (138/148); however, they believed that sheep welfare could be improved on their farms (71.6%) (106/148). When asked about which aspects could be improved, 30.4% (45/148) cited the provision of a better environment, as shelter and comfortable resting areas. In relation to sheep sentience, 52.7% (78/148) agreed and 36.49 (54/148) strongly agreed that sheep are able to clearly distinguish livestock keepers and other people; 54.7% (81/148) agreed and 33.8 (50/148) strongly agreed that sheep feel emotions; 51.3% (76/148) agreed and 29.7% (44/148) strongly agreed that sheep clearly express their feelings. In general, it is possible to observe that the interviewed farmers presented high perception of sheep sentience. On the suffering generated by some practices performed in sheep farming, 39.2% (58/148) and 27.7% (41/148) responded that sheep suffer a little and moderately, respectively, from identification through ear notching or tagging, tattooing or micro-chipping. Regarding castration, 32.4% (48/148) and 31.1% (46/148) cited that sheep present severe to maximum suffering, respectively. In relation to the perception on tail docking, 31.8% (47/148) and 23.0% (34/148), respectively, answered that sheep show moderate and maximum suffering when such practice is performed. Fifty percent (74/148) of farmers responded that sheep do not suffer when sheared. On breeding techniques, different levels of suffering were noted: 24.5% (36/147), 23.8% (35/147) and 21.1% (31/147) responded that sheep show no, moderate and low suffering. When questioned about weaning, 31.8% (47/148) cited that sheep suffer moderately. Farmers showed different levels of perception on sheep suffering from current farming practices. The recognition of sentience and suffering is essential to modify practices that generate low welfare, and to apply legislation to promote sheep welfare. Our results indicate that farmer recognition of suffering due to specific practices needs improvement and may be considered a primary prevention indicator.

Keywords: animal welfare, emotions, opinion.

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# APPENDIX IX – ABSTRACT

## "PERCEPTION OF SHEEP WELFARE AND SENTIENCE BY CITIZENS OF CURITIBA, PARANA, BRAZIL AND CLERMONT-FERRAND, THEIX, FRANCE"

Priscilla Regina Tamioso[1], Daniel Santiago Rucinque[2], Mara Miele[3], Carla Forte Maiolino Molento[4]

[1]Doutoranda do Programa de Pós-graduação em Ciências Veterinárias - PPGCV, Universidade Federal do Paraná - UFPR, Laboratório de Bem-estar Animal - LABEA/UFPR, Campus Agrárias, Curitiba, Paraná. Bolsista da Capes. e-mail: priscillatamioso@gmail.com

[2]Doutorando do Programa de Pós-graduação em Zootecnia, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo FZEA-USP. e-mail: dsrucinqueg@usp.br

[3]Professora da Universidade de Cardiff, Cardiff, Reino Unido. e-mail: mielem@cardiff.ac.uk

[4]Professora associada da Universidade Federal do Paraná - UFPR e coordenadora do Laboratório de Bem-estar Animal - LABEA/UFPR, Campus Agrárias, Curitiba, Paraná. e-mail: carlamolento@ufpr.br

ABSTRACT: We compared the perception of citizens from Curitiba, Parana, Brazil (B) and Clermont-Ferrand, Theix, France (F), concerning sheep welfare and sentience. Animal welfare was defined mainly using terms associated with Freedom from fear and distress, Freedom from hunger, thirst and malnutrition, and Freedom from discomfort. A total of 46.9% B believed that welfare is not taken into consideration for farm animals, in contrast with 3.7% F ($P<0.01$). The consumption of sheep products did not differ between respondents, except for dairy, which was more frequently consumed by F ($P<0.01$). Many F associated animal welfare with physical conditions and showed less perception of sheep sentience than B ($P<0.05$). No significant differences were found for the perception of sheep suffering caused by management practices ($P>0.05$). Mammals were given the highest scores of sentience, and significant differences between B and F were found for pigeon, butterfly, dog, chicken, fish, sheep, cattle and cockroach ($P<0.01$). B and C showed similar definitions of animal welfare, attitudes on the consumption of sheep products and perception of suffering caused by management practices. However, participants differed on their perception of emotions in some species, animal welfare and productivity and sheep emotions; B showed higher perception of animal welfare issues.

Keywords: animal welfare, emotions, opinion, survey

INTRODUCTION

There have been few studies about the society perception in relation to sheep welfare and sentience. In an interview with consumers from European Union countries, the absolute majority of respondents agreed that animals used for food production, including sheep, are able to suffer; additionally, the participants stated that they are not sufficiently informed about the welfare of the animals (Mayfield et al., 2007). It is important to understand citizens' perception of animal welfare and sentience, as citizens participate in political processes. Therefore, our study aimed to

compare the perception of citizens in Curitiba, Parana, Brazil and Clermont-Ferrand, Theix, France, concerning sheep welfare and sentience.

## MATERIAL AND METHODS

Citizens from Curitiba, Parana, Brazil (B) and Clermont-Ferrand, Theix, France (F) were invited to participate in an online survey on Survio® platform from November 2014 to May 2016, in the language spoken in each country. A total of 388 B and 350 F participated in the survey. The questionnaire contained 19 open-end, multiple choices and 5-point Likert-type scale questions, divided into five sections. Demographic questions, as gender, age and education belonged to the first section. The second section comprised questions about animal welfare in general. The next section was composed of questions about consumption of sheep products, contact with sheep, sheep welfare and sentience. The forth section introduced questions about sheep suffering, through different management practices that are commonly performed in the sheep industry. Such questions were presented twice, so that the answers were evaluated according to the respondents' perception when the management practices were presented without descriptions (identification1, castration1, tail docking1, shearing1, reproductive techniques1 and weaning1) and with descriptions of how they are commonly performed (identification2, castration2, tail docking2, shearing2, reproductive techniques2 and weaning2). The last section contained a question about sentience in different species of animals.

In addition to descriptive statistics, we used the non-parametric Mann-Whitney, Kruskal-Wallis and Wilcoxon tests with Minitab software, version 17, at $P<0.05$. Data were analyzed by comparing responses of B and F, considering gender, age and education. The survey comprised a sample with a margin of error equal to 5% and confidence level of 95% for each respondent group. The study was previously approved by the Human Research Ethics Committee of the Federal University of Paraná (Cométicа - SCS/UFPR) under protocol number 814 835/2014.

## RESULTS AND DISCUSSION

Most B defined animal welfare in terms of Freedom from fear and distress (27.0%), Freedom from hunger, thirst and malnutrition (20.5%) and Freedom from discomfort (17.8%). In France, F defined animal welfare comprising Freedom from fear and distress (33.4%), Freedom from discomfort (19.6%) and Freedom from hunger, thirst and malnutrition (19.1%). Similar results were found by Te Velde et al. (2002), who reported that consumers defined animal welfare mostly in terms of physical and mental well-being. Significant differences were found for the consideration of animal welfare, as 3.7% F believe that welfare is not taken into consideration for farm animals, in contrast with 46.9% B ($P<0.01$). Such difference may be due to different animal welfare scenarios in both countries. European countries dispose of a greater availability of labeled welfare-friendly products, consequently, the French consumer may have the idea that farm animals experience good levels of welfare, in addition to the fact that the consumers have more options and more information on the products they buy.

The participants differed on the consumption of dairy, i.e. milk and cheese, as in France, the majority of respondents consumed dairy, at least, a few times a year (36.6%) ($P<0.05$). Significant differences between male and female F were found for the consumption of sheep meat; 22.0% females do not consume sheep meat when compared with 11.0% males ($P<0.05$), in accordance with María et al. (2006). Concerning the consumption of wool, age differences were found for B; lower

consumption was observed among respondents aged 50 years-old or more (69.2%) (P<0.01). Age differences were also found among B for tallow, as all participants aged at 40-49 years-old have never consumed such product (P<0.05). The results show that older citizens from Curitiba tend to consume wool and tallow less frequently. María (2006) also reported significant effect of age on the consumption of animal products among Spanish respondents; however, the author observed that younger people tended to not consume animal products. In addition, significant education differences were found for F. Respondents having secondary or less educational level answered that they have never consumed wool (61.8%), differing from other groups (P<0.01). The findings show that wool is not consumed by citizens from Clermont-Ferrand that have lower educational levels. Results regarding age and educational level are intriguing and require further research.

A total of 21.6% B agreed that sheep that are healthy and grow well have their welfare guaranteed, when compared to 32.9% F (P<0.01) (Fig.1). The result points to higher perception of association between animal welfare and physical conditions by French respondents, which is not in accordance with findings by Phillips & McCulloch (2005). The authors found that Europeans from different nationalities and to some extent students from the USA were more likely to disagree with "the fact that intensively farmed pigs grow well and produce large litters of piglets shows that they are clearly not suffering"; specifically the studied French respondents tended to disagree with the statement (Phillips & McCulloch, 2005). Significant age differences were also found between B and F for such statement; the majority of B aged at 40-49 years-old (34.0%) agreed that sheep that are healthy and grow well have their welfare guaranteed, in comparison with other age classes (P<0.01). A similar result was found for F; most respondents aged at 40-49 (20.6%) and 50 years-old or more (19.05%) strongly agreed with such statement (P<0.01). The results suggest that older citizens tend to view animal welfare mainly in terms of physical health. Respondents from F also differed on their perception according to educational level. Most F with secondary or less educational level (45.6%) agreed with the statement, differing from other categories (P<0.01), indicating that participants with lower educational levels might associate quality of life mainly to physical conditions.

Significant differences were found for the statement "sheep are capable of feeling emotions, such as fear and happiness, in addition to suffering". A total of 75.0% B strongly agreed with the statement, in contrast with 66.3% F (P<0.05) (Fig.1). The fact that less participants in France strongly agreed that sheep are capable of feeling emotions is an interesting result, as in Clermont-Ferrand there are important sheep producers; consequently, we would expect that people would be more familiar to sheep and would better recognize their emotional capacities. Female F also showed higher perception of sheep emotions, as 70.6% strongly agreed that sheep feel emotions, in contrast with 59.5% males (P<0.05), in agreement with other findings that show that women have greater concern and empathy toward animal welfare and sentience (María et al., 2006).

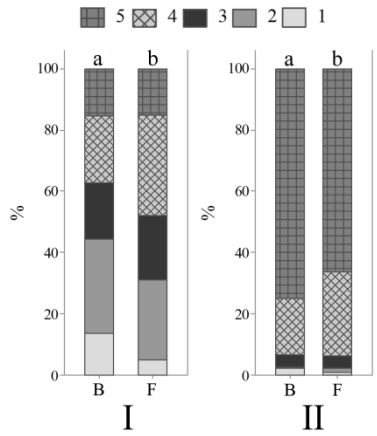


Figure 1. Levels of agreement concerning sheep welfare and sentience, by 388 citizens from Curitiba, Parana, Brazil (B) and 350 citizens from Clermont-Ferrand, Theix, France (F); November 2014 to May 2016; (I) Sheep that are healthy and grow well have their welfare guaranteed; (II) Sheep are capable of feeling emotions, such as fear and happiness, in addition to suffering; 1 = strongly disagree; 2 = disagree; 3 = neutral/unsure; 4 = agree; 5 = strongly agree; letters indicate differences between B and F ($P<0.05$, Mann-Whitney test).

No significant differences were found between B and F regarding suffering caused by management practices ($P>0.05$), indicating that the surveyed participants have similar perceptions of sheep suffering. However, gender had significant influence over participants' perceptions. Female B increased their perception from moments 1 and 2 of suffering during identification, castration, tail docking, reproductive techniques and weaning ($P<0.05$) (Fig.2). Among F, increasing perception between moments 1 and 2 was found for tail docking, reproductive techniques and weaning among women and, among men, for reproductive techniques and weaning ($P<0.05$) (Fig.2). Furthermore, in general, women showed higher perception of sheep suffering than men ($P<0.05$) (Fig.2). These results were expected, as women tend to react more emotionally and empathetically to animal suffering.

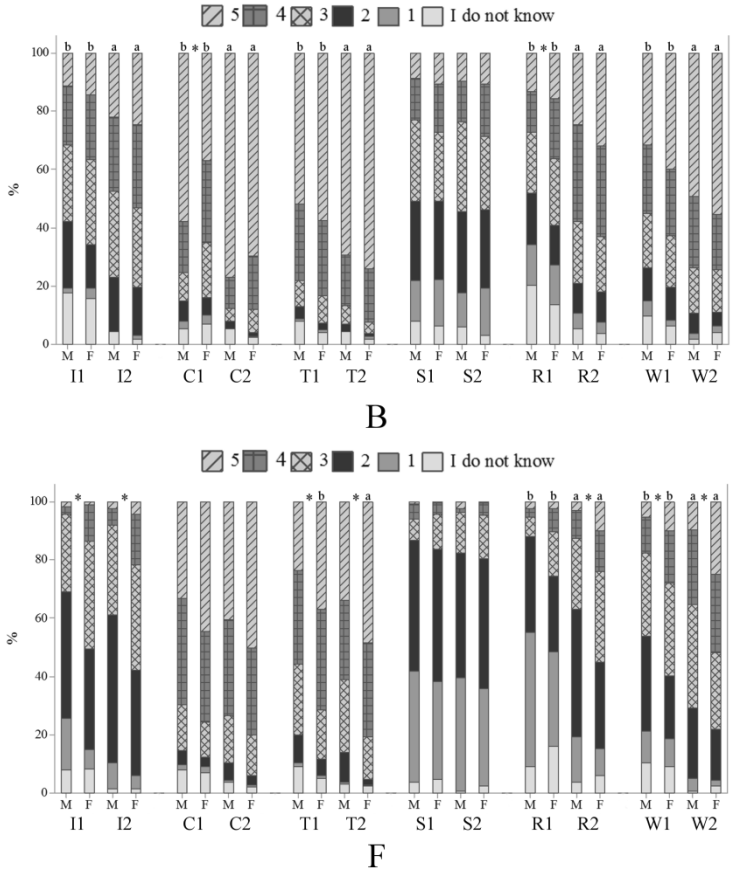


Figure 2. Levels of suffering attributed to different management practices by 388 citizens from Curitiba, Parana, Brazil (B) and 350 citizens from Clermont-Ferrand, Theix, France (F); November 2014 to May 2016; M = male; F = female; 1 = no suffering; 2 = mild suffering; 3 = moderate suffering; 4 = severe suffering; 5 = very severe suffering; I1 = identification1; I2 identification2; C1 = castration1; C2 = castration2; T1 = tail docking1; T2 = tail docking2, S1 = shearing1; S2 = shearing2; R1 = reproductive techniques1; R2 reproductive techniques2; W1 = weaning1; W2 = weaning2; letters indicate gender differences between the first and second moments of each management practice ($P<0.05$; Wilcoxon test); asterisks indicate significant differences between male and female respondents ($P<0.05$; Mann-Whitney test).

Fig.3 shows that mammals were given the highest scores by the participants, followed by birds, fish and invertebrates. Significant differences between B and F were found for pigeon, butterfly, dog, chicken, fish, sheep, cattle and cockroach ($P<0.01$) (Fig.3), as B attributed higher scores of emotions to such animals. The fact that B attributed higher scores of sentience to specific animals may be associated with lower fear of such animals and more experience with them. Experience with animals seems to be positively related to positive attitudes to them (Morris et al., 2012).

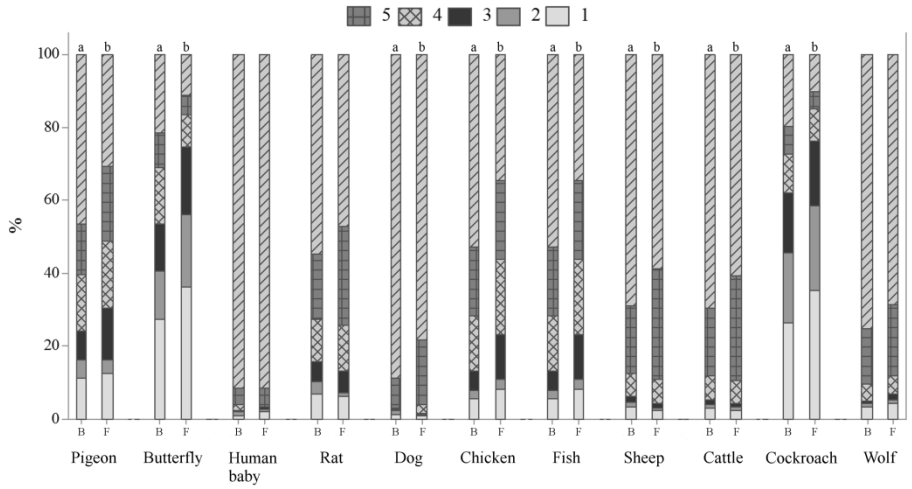


Figure 3. The ability of different animals to feel emotions, in a scale from 1 to 5, being 1 the animal does not feel emotions, 5 the animal certainly feels emotions and intermediate values are equivalent to a growing capacity to feel emotions, according to 388 citizens from Curitiba, Parana, Brazil (B) and 350 citizens from Clermont-Ferrand, Theix, France (F); November 2014 to May 2016; letters indicate differences between B and F ($P<0.05$, Mann-Whitney test).

Significant gender differences were also noted for perception of sentience in cattle, sheep and cockroach by F, as females attributed the highest scores (65.55%; 64.1%; 42.3%, respectively) ($P<0.05$). Again, these results were expected. The perception of sentience in some species also differed according to the age groups, only in B. A total of 40.0% B aged at 40-49 years-old attributed the highest scores of sentience to pigeons, in contrast with 53.3% participants aged at 18-29 years-old ($P<0.05$). Additionally, 31.7% B aged 40-49 years-old scored the highest level of emotions to fish, in comparison with other age groups ($P<0.05$). Concerning the cockroach, 42.4% B aged at 50 years-old or more attributed the highest rate of emotional states to such animal, differing statistically from the other age classes ($P<0.05$). The results suggest a positive correlation between age and interest in animals, as older B seemed to show more interest, empathy toward specific animals. Lastly, a significant effect of education was also noted among B, for some animals. The majority of B having secondary or less educational level attributed the highest scores of emotions to pigeon (33.3%), chicken (38.2%) and sheep (51.35%), differing from other groups ($P<0.05$). Contrary to our findings, Bjerke & Østdahl (2014) found a positive association between education and scores of preference for the majority of animals, as the preference scores increased with increasing educational levels, with exception of dogs, cats and rats, among some others.

This is the first study to show the effect of demographic variables on the perceptions of Brazilian and French respondents regarding animal sentience and comparisons amongst these perceptions. The results indicate that there is higher perception of emotional capacities for specific animals; therefore, more studies are necessary to be conducted.

## CONCLUSIONS

Citizens from Curitiba and Clermont-Ferrand showed similar perceptions of the definition of animal welfare. They also showed similar attitudes on the consumption of sheep products, except to dairy, and perception of suffering caused by management practices. However, participants differed on their perception of

emotions in some species, animal welfare and productivity and sheep emotions; citizens from Curitiba showed higher perception of animal welfare issues. The evaluation of emotions by different sectors of the population may contribute to the recognition that sheep are sentient beings and, consequently, such recognition may lead to improvements in their quality of life.

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# APPENDIX X – ABSTRACT

# "PERCEPTION OF SHEEP WELFARE AND SENTIENCE BY CITIZENS, VETERINARIANS, BIOLOGISTS AND ANIMAL SCIENTISTS OF CURITIBA, PARANA, BRAZIL"

Priscilla Regina Tamioso[1], Daniel Santiago Rucinque[2], Mara Miele[3], Carla Forte Maiolino Molento[4]

[1]Doutoranda do Programa de Pós-graduação em Ciências Veterinárias - PPGCV, Universidade Federal do Paraná - UFPR, Laboratório de Bem-estar Animal - LABEA/UFPR, Campus Agrárias, Curitiba, Paraná. Bolsista da Capes. e-mail: priscillatamioso@gmail.com

[2]Doutorando do Programa de Pós-graduação em Zootecnia, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo FZEA-USP. e-mail: dsrucinqueg@usp.br

[3]Professora da Universidade de Cardiff, Cardiff, Reino Unido. e-mail: mielem@cardiff.ac.uk

[4]Professora associada da Universidade Federal do Paraná - UFPR e coordenadora do Laboratório de Bem-estar Animal - LABEA/UFPR, Campus Agrárias, Curitiba, Paraná. e-mail: carlamolento@ufpr.br

ABSTRACT: We compared the perception of citizens (C), veterinarians (V), biologists (B) and animal scientists (A) from Curitiba, Parana, Brazil, regarding sheep welfare and sentience. Knowledge about animal welfare in C (15.2%) differed from V (0.0%), B (1.1%) and A (0.0%), in terms of respondents who did not know about the subject ($P<0.01$). Animal welfare was defined mainly considering terms related to Freedom from fear and distress, Freedom from hunger, thirst and malnutrition and Freedom from discomfort. C and B differed from V and A on the perception of farm animal welfare, as C and B believed that welfare is not or less considered for farm animals ($P<0.05$). In addition, C and V showed higher perception of association between higher levels of animal welfare and productivity than B and A ($P<0.05$). The perception of sheep sentience did not differ among respondents ($P>0.05$). When asked about sheep suffering caused by management practices, in general, V and A attributed lower scores of suffering, when compared to C and B ($P<0.05$). The results suggest that C and B, and V and A, have similar perceptions on the consideration of welfare for farm animals and sheep suffering. The respondents showed similar perceptions of sheep sentience.

Keywords: attitudes, animal welfare, human-animal interaction, suffering

## INTRODUCTION

It has been reported that the attribution of emotional experiences to animals is directly associated with a positive treatment towards them (Knight et al., 2004). Combined with scientific studies on affective states and cognition in farm animals, the recognition that they are sentient beings may increase the importance and acceptance of the need to prioritize their welfare. This way, it is important to understand citizens' perception of animal welfare and sentience, as they participate in political processes. In addition, research on the perception of different professionals who interact with animals is essential, as such professionals are

directly involved in issues associated with animal welfare, are commonly involved in decisions that affect animals and may contribute to spread information on animal welfare to several sectors of the society, such as citizens, consumers, farmers and stockpeople. Therefore, our study aimed to compare the perception of citizens and different professionals who interact with animals from Curitiba, Parana, Brazil, toward sheep welfare and sentience.

## MATERIAL AND METHODS

Respondents from Curitiba, Parana, Brazil were invited to participate in an online survey on Survio® platform from November 2014 to May 2016. The study population was divided in four categories: citizens (C), veterinarians (V), biologists (B) and animal scientists (A). From a total of 986 respondents, 753 were selected, as they lived in Curitiba, being 388 C, 248 V, 92 B and 25 A. The survey comprised a sample with a margin of error equal to 5% and confidence level of 95% for each respondent category. The study was previously approved by the Human Research Ethics Committee of the Federal University of Paraná (Cométicа - SCS/UFPR), under protocol number 814 835/2014.

The study comprised questions on animal welfare, sheep welfare and sentience (Table 1). Data were analyzed using descriptive statistics and by comparing responses of C, V, B and A. Non-parametric Kruskal-Wallis and Wilcoxon tests were used at $P<0.05$, through Minitab software, version 17.

Table 1. Main questions (Q) available to 388 citizens (C), 248 veterinarians (V), 92 biologists (B) and 25 animal scientists (A) from Curitiba, Parana, Brazil; November 2014 to May 2016.

| Questions | Content | Options of answers |
|---|---|---|
| Q01 | Have you ever heard of animal welfare? | Yes, I know what animal welfare is; Yes, I know the subject superficially; No, I have never heard of animal welfare. |
| Q02 | If yes, what do you think animal welfare consists of? | Open question. |
| Q03 | Do you think welfare is taken into consideration for farm animals? | Yes, fully; Yes, most of the times; Yes, half of the times; Yes, a few times; No, never; I do not know. |
| Q04 | In a scale from 1 to 5, please select the rating that best describes your opinion:<br>Sheep that are healthy and grow well have their welfare guaranteed.<br>Sheep are capable of feeling emotions, such as fear and happiness, in addition to suffering. | 1 strongly disagree; 2 disagree; 3 neutral/unsure; 4 agree; 5 strongly agree. |
| Q05 | In a scale from 1 to 5, classify the management practices that are frequently performed on sheep farms according to your perception of sheep suffering: identification1, castration1, tail docking1, shearing1, reproductive techniques1 and weaning1. | 1; 2; 3; 4; 5; I do not know 1 no suffering; 2 mild suffering; 3 moderate suffering; 4 severe suffering; 5 very severe suffering. |

| | | |
|---|---|---|
| Q06 | The same management practices from the previous question are described below, with definitions on how they are commonly performed. Rate them again according to your perception of sheep suffering: Identification2: through ear notching or punching, tattooing, ear tagging or micro-chipping. Castration2: removal or destruction of the testicles, through rubber rings, emasculator/burdizzo or surgery. Tail docking2: through rubber rings, cauterization using a hot docking iron or surgery. Shearing2: cutting or shaving the fleece/wool, though the use of electric shears, shearing machines or scissors. Reproductive techniques2: artificial insemination, synchronization of estrus (through the use of intravaginal sponge impregnated with progestagen) and laparoscopic embryo transfer. Weaning2: separation of ewes and lambs before the lambs reach 6 months of age. | 1; 2; 3; 4; 5; I do not know 1 no suffering; 2 mild suffering; 3 moderate suffering; 4 severe suffering; 5 very severe suffering. |
| Q07 | In a scale from 1 to 5, classify the ability of each animal to feel emotions: pigeon, butterfly, human baby, rat, dog, chicken, fish, sheep, cattle, cockroach and wolf. | 1; 2; 3; 4; 5; I do not know 1 the animal does not feel emotions; 5 the animal certainly feels emotions; intermediate values are equivalent to a growing capacity to feel emotions. |

## RESULTS AND DISCUSSION

Citizens differed from veterinarians, biologists and animal scientists in their knowledge about animal welfare. A total of 15.2% C responded that they have never heard of animal welfare, in contrast with 0% V, 1.1% B and 0% A ($P<0.01$), which might be explained by the fact that the topic is studied by the surveyed professionals. Schnettler et al. (2008) also found that 17% of the consumers in Chile stated that they do not have knowledge about animal welfare.

Most C defined animal welfare in terms of Freedom from fear and distress (27.0%), Freedom from hunger, thirst and malnutrition (20.5%) and Freedom from discomfort (17.8%). Freedom from fear and distress was acknowledged 24.8% of the times by V and 25.9% by B, Freedom from hunger, thirst and malnutrition was mentioned 20.9% of the times by V and 23.1% by B and Freedom from discomfort, 18.5% by V and 17.0% by B. Terms related to Freedom from fear and distress (21.9%) and Freedom from hunger thirst and malnutrition (18.8%) were mostly acknowledged by A. Aspects related to animal nutrition, animal health and human-animal relationship, in addition to environmental aspects, animal suffering and stress, were also acknowledged by Belgian respondents in a study by Vanhonacker et al. (2008).

A total of 46.9% C and 29.3% B believed that welfare is not taken into consideration for farm animals, in comparison with 18.5% V and 12.0% A ($P<0.01$). Higher concern by citizens and biologists may be related to the fact that they are not used to interact with farm animals, as veterinarians and animal scientists do; the latter, being used to management practices and farming systems, may end up banalizing the scenario faced by farm animals and considering it normal.

When asked if sheep that are healthy and grow well have their welfare guaranteed, 15.5% C and 11.3% V strongly agreed with the statement, differing from

6.5% B and 4.0% A (P<0.05) (Fig. 1). It was expected that professionals that interact with farm animals, mainly veterinarians and animal scientists, would have a similar perception, therefore further studies are necessary to better understand such finding. In a survey with students of a veterinary faculty, 40% agreed that if animals are producing (e.g. gaining weight or producing eggs) they have good welfare (Heleski et al., 2005). No differences were found among C, V, B and A for the percetion on sheep sentience (P>0.05); in general, most of respondents agreed or strongly agreed that sheep experience emotions.

When the perception of suffering caused by management practices that are commonly performed in the sheep industry was compared, the perception of identification1 differed significantly. A total of 16.1% C believed that sheep suffer very severely, in contrast with 2.5% V, 8.6% B and 12.0% A (P<0.01) (Fig.1). Lower consideration toward suffering in management practices by the professionals might be due to loss of sensitivity in the end of graduation, which might persist during the professional life. A total of 74.1% C believed that sheep suffer very severely in castration2, in contrast with 52.5% V, 64.1% B and 64.0% A (P<0.01) (Fig.1). Higher concern about sheep suffering by citizens might be due to the fact that this group may be more sensitive toward farming practices, as the other categories are more exposed to common practices in livestock industry. The perception of tail docking1 was the lowest by V, as 41.1% believed that sheep show very severe suffering, in contrast with 58.7% C, 50.7% B and 60.0% A (P<0.01) (Fig.1). The perception of tail docking2 was higher by C (74.6%) and B (71.7%) than by V (52.65%) and A (52.0%) (P<0.01) (Fig.1). The groups also differed on their perception to shearing1. A total of 10.8% C and 3.3% B claimed that sheep suffer very severely when sheared, in contrast with 1.6% V and 4.2% A (P<0.01) (Fig.1). For shearing 2, similar results were found; higher perception of suffering was found by C (10.7%) and B (4.4%), when compared to V (1.2%) and A (4.0%) (P<0.01) (Fig.1). Significant differences were noted for reproductive techniques1: C (17.7%) and B (28.1%) showed higher perception of suffering in sheep, than V (4.2%) and A (0.0%) (P<0.01) (Fig.1); and reproductive techniques2: C (31.0%) and B (9.5%) believed that sheep suffer very severely, than V (11.0%) and A (0.0%) (P<0.01) (Fig.1). Weaning 1 and 2 were also perceived differently. A total of 40.3% C attributed the highest level of suffering for weaning1 (P<0.01), differing from B (32.9%), V (24.4%) and A (20.0%) (P<0.01) (Fig.1). For weaning2, C and B differed from V and A; 55.5% C and 44.9% B believed that sheep suffer very severely, in comparison with 33.1% V and 20.0% A (P<0.01) (Fig.1). In general, the respondents attributed some level of suffering to sheep due to management practices. In addition, C and B showed similar perceptions of sheep suffering due to management practices, as well as V and C.

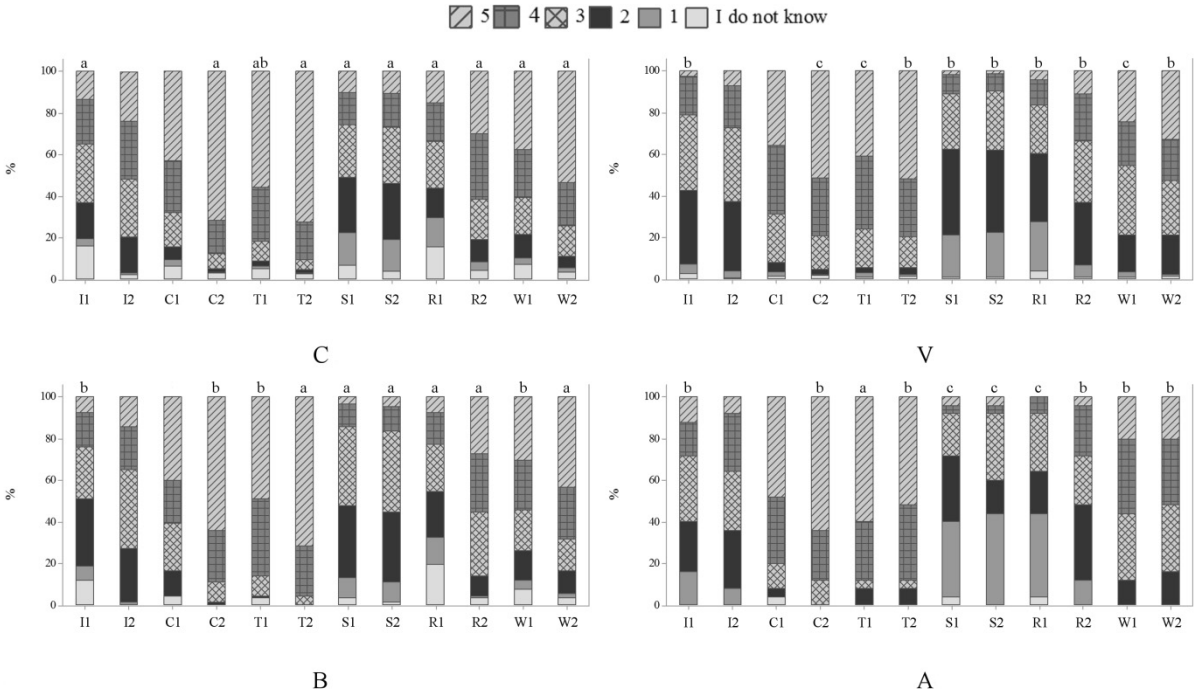


Figure 1. Levels of suffering attributed to different management practices (Q05-Q06) by 388 citizens (C), 248 veterinarians (V), 92 biologists (B) and 25 animal scientists (A) from Curitiba, Parana, Brazil; November 2014 to May 2016; 1 = no suffering; 2 = mild suffering; 3 = moderate suffering; 4 = severe suffering; 5 = very severe suffering; I1 = identification1; I2 identification2; C1 = castration1; C2 = castration2; T1 = tail docking1; T2 = tail docking2, S1 = shearing1; S2 = shearing2; R1 = reproductive techniques1; R2 reproductive techniques2; W1 = weaning1; W2 = weaning2; letters indicate differences between respondents for each management practice (P<0.05, Kruskal-Wallis test).

Mammals were given the highest scores of emotional capacities and invertebrates, the lowest (Fig.2). Significant differences were found among respondent groups for some animals; a total of 29.4% C showed the highest perception of sentience to butterfly, compared with 19.2% V, 29.5% B and 15.0% A (P<0.05) (Fig.2). As butterflies are commonly attributed some aesthetic appeal, compared to other invertebrates, it was expected that they were given higher levels of sentience by all the respondents. On the opposite, 74.2% B showed the highest perception toward rats, differing from the other groups (P<0.01) (Fig.2). Mice are usually rated the lowest in preference/empathy ranks, due to the fear appeal and low concern, as they are known to spread diseases (Borgi & Cirulli, 2015). However, higher perception of sentience in rats by biologists may be due to interactions and familiarity with such animals during the graduation course, for example. The attribution of higher emotional capacities to specific animals by the respondents suggests the necessity of more studies to better understand the results.

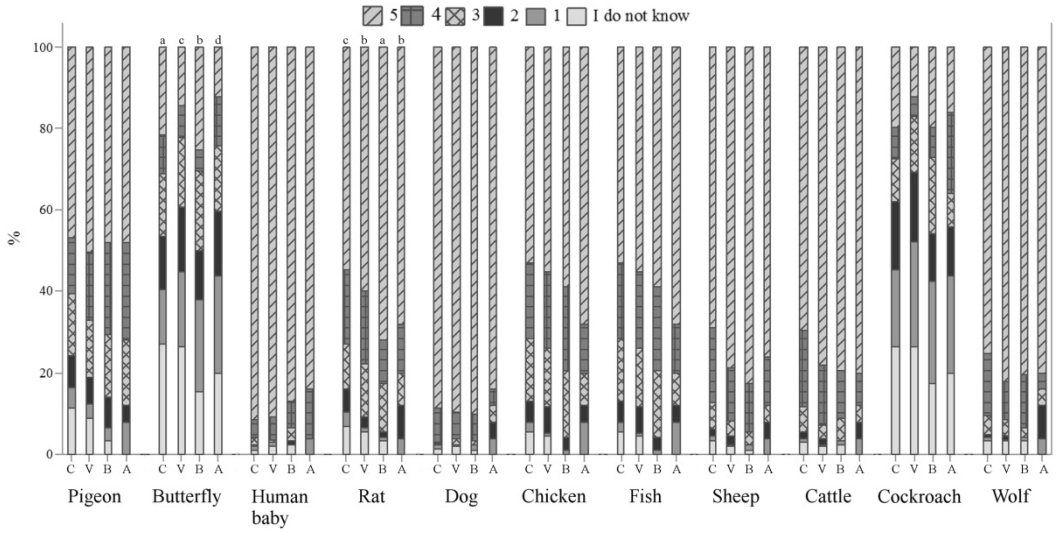


Figure 2. The ability of different animals to feel emotions (Q07), in a scale from 1 to 5, being 1 the animal does not feel emotions, 5 the animal certainly feels emotions and intermediate values are equivalent to a growing capacity to feel emotions, according to 388 citizens (C), 248 veterinarians (V), 92 biologists (B) and 25 animal scientists (A) from Curitiba, Parana, Brazil; November 2014 to May 2016; letters indicate differences between respondents ($P<0.05$, Kruskal-Wallis test).

## CONCLUSIONS

The results suggest that citizens and biologists, as well as veterinarians and animal scientists, have similar perceptions on the consideration of welfare for farm animals and suffering caused to sheep due to specific management practices. The respondents showed similar perceptions of the emotional capacities of sheep. This is the first time that differences in the perception of animal welfare issues between citizens, veterinarians, biologists and animal scientists are observed in Brazil. The knowledge presented may guide specific initiatives to improve perceptions, as well as future research.

## ACKNOWLEDGEMENTS

The project was funded by a grant to the first author from the Coordination for the Improvement of Higher Education Personnel (Capes). The authors are grateful to the participants, as well as the Regional Council of Veterinary Medicine, the Regional Council of Biology of the State of Parana and ADAPAR.

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# APPENDIX XI – ABSTRACT

# "PERCEPÇÃO DE CIDADÃOS DA COLÔMBIA E DO BRASIL EM RELAÇÃO À SENCIÊNCIA EM DIFERENTES GRUPOS DE ANIMAIS"

Daniel Santiago Rucinque
Priscilla Regina Tamioso
Carla Forte Maiolino Molento
Laboratório de Bem-estar Animal – LABEA, Universidade Federal do Paraná – UFPR. Rua dos Funcionários, 1540. CEP: 80035-050, Juvevê, Curitiba/PR, Brasil.
Autora para correspondência: carlamolento@ufpr.br

RESUMO: A senciência animal é definida pela capacidade de experimentar emoções associadas à consciência. O objetivo do presente estudo foi comparar a percepção de cidadãos de Bogotá D.C./Colômbia (B) e Curitiba-PR/Brasil (C) em relação à senciência em diferentes grupos de animais. Um questionário online foi disponibilizado às populações de Bogotá D.C. (B) e Curitiba-Paraná (C), em seus respectivos idiomas, sobre a percepção de senciência em 11 grupos de animais. Os respondentes foram convidados a atribuir uma nota, a partir de escala de 1 a 5, acerca da capacidade de sentir emoções. A classificação descendente de senciência percebida ($p<0,05$) para os grupos de animais foi bebê humano, cachorro, lobo, boi, ovelha, rato, galinha, pombo, peixe, borboleta e barata. Os resultados sugerem uma atribuição maior de senciência em ambas as cidades em relação aos mamíferos. Os cidadãos de Curitiba-PR apresentam de forma geral uma maior percepção de senciência quando comparados aos cidadãos de Bogotá D.C. O reconhecimento da senciência é fundamental para modificar legislação e políticas que promovam melhorias no grau de bem-estar dos animais utilizados em diferentes cenários.
Palavras-chave: bem-estar animal, estados emocionais, opinião.

## INTRODUÇÃO

O reconhecimento da senciência animal é fundamental para modificar normas e políticas de proteção animal no sentido de evitar atos de maus-tratos. Webster (2006) definiu um animal senciente como aquele que experimenta emoções associadas a sentimentos de prazer e sofrimento e que apresenta motivações comportamentais de origem evolutiva (1). Neste sentido, os animais cientificamente considerados sencientes pertencem ao grupo dos vertebrados e cefalópodes (2). Em países europeus, cidadãos exigem padrões crescentes de bem-estar animal, e suas opiniões tendem a se tornar diretrizes (3).

Na América Latina, o conhecimento sobre a percepção da população em relação à senciência animal é ainda escasso. O objetivo do presente estudo foi comparar a percepção de cidadãos de Bogotá D.C./Colômbia (B) e Curitiba-PR/Brasil (C) em relação à senciência em diferentes grupos de animais.

## MATERIAL E MÉTODOS

Um questionário online foi disponibilizado às populações de Bogotá D.C. (B) e Curitiba-Paraná (C), em seus respectivos idiomas, com o objetivo de avaliar noções gerais de percepção sobre senciência em diferentes grupos animais. Os respondentes foram convidados a atribuir uma nota em relação à capacidade de

sentir emoções a 11 grupos de animais, sendo que: 1 o animal não sente emoções e 5 o animal certamente sente emoções, em que os valores intermediários significavam capacidade crescente de sentir. Adicionalmente foi fornecida a opção “eu não sei”.

As respostas foram coletadas do período de agosto de 2014 a março de 2015. A fim de comparar as cidades B e C, utilizou-se teste de Mann-Whitney ao nível de significância de 5%. O teste Kruskal-Wallis seguido do teste de Dunn foi utilizado para comparar as notas atribuídas os grupos de animais. As análises foram feitas no programa Minitab versão 17. Após selecionar somente os respondentes das cidades de interesse, foram avaliadas respostas de 395 participantes de Bogotá D.C. e de 415 de Curitiba-PR. O estudo foi aprovado no Comitê de Ética em Pesquisa do Setor de Ciências na Saúde da UFPR, sob o número de protocolo 814835/2014.

## RESULTADOS E DISCUSSÃO

A distribuição das notas atribuídas em cada grupo de animais nas duas cidades encontram-se na Figura 1. A classificação descendente de percepção de senciência nos grupos de animais foi bebê humano, cachorro, lobo, boi, ovelha, rato, galinha, pombo, peixe, borboleta e barata. A classificação mostra que existe uma maior sensibilidade com os animais mais próximos filogeneticamente, neste caso os mamíferos. Além disso, animais mais distantes como aves, peixes e invertebrados são percebidos com um menor grau de senciência, questão também mencionada por Broom (2007) (4). Das notas atribuídas aos 11 grupos de animais, 10 apresentaram diferença significativa ($p<0,05$) evidenciando uma atribuição maior de senciência em cidadãos de Curitiba-PR. As diferenças podem estar relacionadas às características de avanço na legislação de proteção animal de cada país. Na Colômbia, a legislação encontra-se desatualizada desde 1989, e ainda são permitidas rinhas de galo, corridas de touros, entre outras práticas consideradas como maus-tratos. No Brasil rinhas de galo e corridas de touros, e outras práticas, são proibidas desde 1994 (5).

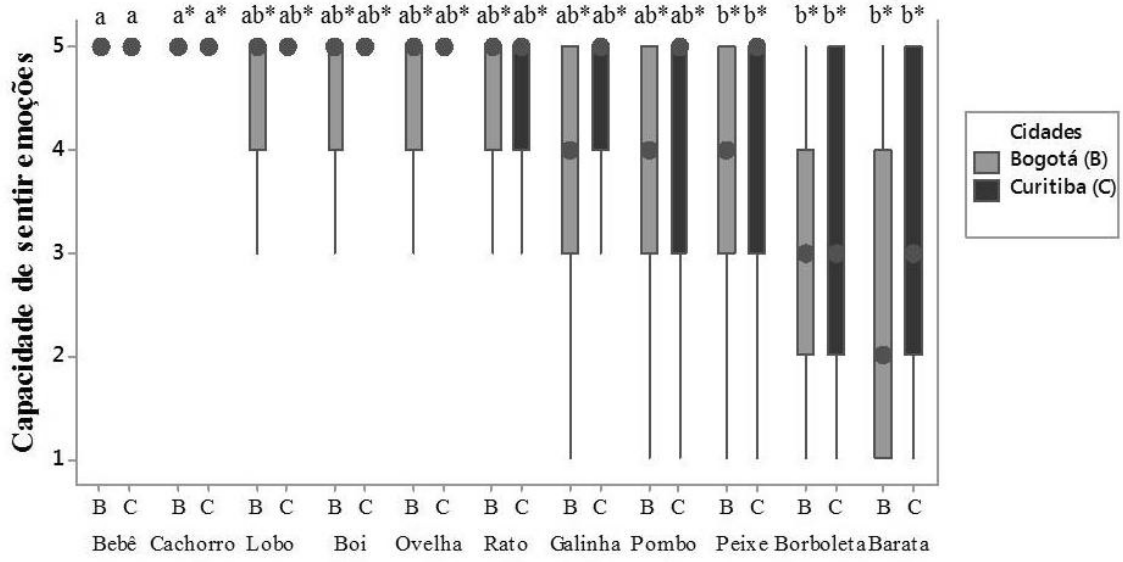


Figura 1. Medianas das notas atribuídas à capacidade de sentir emoções, em diferentes grupos de animais por cidadãos de Bogotá D.C. e Curitiba-PR. Sendo que: 1 = o animal não sente emoções e 5 = o animal certamente sente emoções, valores intermediários significavam capacidade crescente de sentir. Letras diferentes indicam diferença entre grupos de animais ($p<0,05$, Kruskal-Wallis e Teste Dunn). O asterisco indica diferença entre as cidades ($p<0,05$, Mann-Whitney).

## CONCLUSÕES

Os resultados sugerem uma maior percepção de senciência em animais pertencentes à classe dos mamíferos. Em geral, os cidadãos de Curitiba-PR apresentaram uma maior percepção de senciência quando comparados aos cidadãos de Bogotá D.C. Os resultados sugerem a necessidade de fomentar uma melhoria na percepção da população em relação à capacidade de sofrer de animais menos próximos do ser humano como aves e peixes.

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## APPENDIX XII – ABSTRACT

## "PERCEPÇÃO DA POPULAÇÃO CURITIBANA EM RELAÇÃO A ESTADOS EMOCIONAIS EM OVINOS: RESULTADOS PRELIMINARES"

Priscilla Regina Tamioso
Vanessa Carli Bones
Carla Forte Maiolino Molento
Laboratório de Bem-estar Animal – LABEA, Universidade Federal do Paraná – UFPR. Rua dos Funcionários, 1540. CEP: 80035-050, Juvevê, Curitiba/PR, Brasil.
Autora para correspondência: carlamolento@yahoo.com

RESUMO: Relatos acerca da senciência animal contribuem para o interesse da população em relação a questões éticas e de bem-estar. Objetivou-se estudar a percepção de respondentes que interagem com animais (IA), como médicos veterinários, zootecnistas e biólogos, e cidadãos comuns (CC), de Curitiba-Paraná, quanto a estados emocionais em ovinos. Em um questionário online, foram disponibilizados três vídeos com ovinos em situações que eliciavam estados emocionais de valências positiva e negativa. Cada vídeo foi apresentado em duas situações; na primeira, para a descrição do estado emocional do animal e, na segunda, para escolha entre dez opções de adjetivos apresentados. Os resultados foram avaliados por estatística descritiva e pelo teste qui-quadrado. A maioria dos respondentes reconheceu a valência correta das situações apresentadas, sem diferenças significativas entre os grupos. Além disso, os descritores utilizados nas perguntas abertas e fechadas foram semelhantes entre os segmentos IA e CC. A avaliação de emoções por diferentes setores da população poderá contribuir para o reconhecimento de que ovinos são sencientes e dotados de capacidades emocionais.

Palavras-chave: bem-estar animal, opinião, senciência, vídeos.

### INTRODUÇÃO

Pesquisas que relatam a existência de senciência em animais contribuem para um crescente interesse em relação a questões éticas e de qualidade de vida animal. Animais submetidos ao manejo extensivo, como ovinos, geralmente não recebem atenção significativa da sociedade, uma vez que o sistema de produção gera a ideia de que são criados de maneira natural, livres e, assim, experimentam níveis adequados de bem-estar animal (1). Ovinos também são geralmente retratados como animais com menores habilidades mentais e cognitivas, quando comparados a cães, gatos, cavalos, suínos e bovinos (2). Entretanto, o conhecimento quanto à opinião da população refere-se especialmente a pesquisas europeias e norte-americanas. O objetivo deste trabalho foi estudar a percepção de emoções em ovinos por médicos veterinários, zootecnistas e biólogos, e cidadãos comuns, no município de Curitiba.

### MATERIAL E MÉTODOS

Os participantes foram convidados a responder um questionário online contendo 24 perguntas abertas e fechadas sobre bem-estar animal e senciência, com enfoque em ovinos. Tal questionário, previamente aprovado pelo Comitê de Ética em Pesquisa com Seres Humanos (Cométicа – UFPR) sob parecer - CEP/SD:

814 835, foi disponibilizado a partir de novembro de 2014, contando com 606 respondentes até março de 2015. Foram avaliados dois grupos de respondentes: profissionais que interagem diretamente com animais, como médicos veterinários, zootecnistas e biólogos (IA), e cidadãos comuns (CC), de Curitiba-Paraná. Após seleção, foram analisadas 110 respostas da categoria IA, sendo destes 50 médicos veterinários, 43 biólogos e 17 zootecnistas, e 304 da categoria CC, totalizando 414 participantes.

Na plataforma online, foram disponibilizados três vídeos de até 50 segundos com ovinos em situações que eliciavam diferentes estados afetivos. O primeiro vídeo exibia um cordeiro explorando área de pasto e expressando comportamento lúdico (valência positiva); o segundo, um cordeiro em isolamento total, em baia não familiar (valência negativa); e o terceiro, um borrego recebendo escovação (valência positiva). Cada vídeo foi apresentado em dois momentos; no primeiro, os respondentes descreveram o estado emocional do animal em até três adjetivos e, no segundo, foram apresentadas 10 opções de descritores de conotação emocional de diferentes valências, adaptados do protocolo Qualitative Behaviour Assessement – QBA®, para que os respondentes escolhessem os que mais bem representassem as prováveis emoções.

Para a quantificação dos descritores de estados emocionais, foi aplicada estatística descritiva. Após, foi realizada uma comparação de valências positiva e negativa entre os grupos IA e CC por meio do teste qui-quadrado.

## RESULTADOS E DISCUSSÃO

A maioria dos respondentes de ambos os grupos percebeu as situações exibidas nas filmagens de acordo com a valência correta. Entretanto, um porcentual importante não foi capaz de identificar a valência emocional. No primeiro vídeo, para a pergunta aberta, observou-se que 67,3% e 67,8% dos respondentes reconheceram a valência positiva das categorias IA e CC, e para a fechada, 68,2% e 71,9% para IA e CC, respectivamente. No segundo vídeo, 90% dos IA e 90% dos CC avaliaram a situação como negativa em ambos os formatos de pergunta. Por fim, no vídeo 3 a situação foi avaliada como positiva por 80,2% e 79,7% e 74,5% e 74,1% dos respondentes das categorias IA e CC, para as perguntas aberta e fechada, respectivamente. A diferença não foi significativa ($p>0,05$) quando comparadas as opiniões em termos de valências positiva e negativa entre grupos, ou seja, os respondentes IA e CC apresentaram percepções semelhantes, resultado curioso, uma vez que se esperava maior frequência de percepção correta da valência por respondentes IA.

A Tabela 1 contém uma lista de descritores usados pelos grupos de respondentes nas questões abertas e fechadas. Foram selecionados os três adjetivos mais citados pelos respondentes, em total de 49, 35 e 33, do grupo IA e 107, 67 e 98 descritores diferentes, do grupo CC, para os vídeos 1, 2 e 3, respectivamente. Conforme esperado, observa-se maior variância dos descritores em relação às perguntas abertas.

Tabela 1. Frequência absoluta (FA) e percentagem (%) de adjetivos mais citados pelos respondentes das categorias profissionais que interagem com animais (IA) e cidadão comum (CC), para as perguntas abertas e fechadas em relação aos vídeos 1, 2 e 3.

| Valência/ vídeo | Pergunta aberta | | | | Pergunta Fechada | | | |
|---|---|---|---|---|---|---|---|---|
| | IA | | CC | | IA | | CC | |
| | Adjetivo | FA (%)[1] | Adjetivo | FA (%) | Adjetivo | FA (%) | Adjetivo | FA (%) |
| Positiva/ vídeo 1 | Feliz | 42 (41,6) | Feliz | 121 (39,8) | Curioso | 82 (80,2) | Alegre | 210 (69,1) |
| | Livre | 19 (18,8) | Livre | 65 (21,4) | Alegre | 81 (80,2) | Curioso | 172 (56,6) |
| | Curioso | 14 (13,9) | Alegre | 46 (15,1) | Calmo | 52 (51,5) | Calmo | 116 (38,1) |
| Negativa/ vídeo 2 | Com medo | 33 (32,7) | Com medo | 72 (23,7) | Assustado | 70 (69,3) | Assustado | 208 (68,4) |
| | Assustado | 25 (24,7) | Sozinho | 60 (19,7) | Com medo | 69,0 (68,3) | Com medo | 185 (60,9) |
| | Sozinho | 24 (23,8) | Triste | 50 (16,4) | Estressado | 60 (59,4) | Estressado | 178 (58,6) |
| Positiva/ vídeo 3 | Tranquilo | 37 (36,6) | Tranquilo | 70 (23,0) | Calmo | 99 (98,0) | Calmo | 253 (83,2) |
| | Confortável | 26 (25,7) | Feliz | 48 (17,8) | Alegre | 56 (55,4) | Alegre | 131 (40,0) |
| | Relaxado | 20 (19,8) | Calmo | 39 (12,8) | Dominante | 10 (9,9) | Dominante | 31 (10,2) |

1- A sobreposição é maior na pergunta fechada, porque os descritores na aberta já estão pré-definidos e se encontram em número limitado.

## CONCLUSÃO

O porcentual de acerto entre profissionais que interagem com animais e cidadãos comuns foi similar, entretanto, cabe melhorar a percepção de ambos os segmentos acerca do reconhecimento da valência correta das emoções em ovinos. A avaliação de emoções por diferentes setores da população poderá contribuir para o reconhecimento de que ovinos são seres sencientes e, consequentemente, melhorar sua qualidade de vida.

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## APPENDIX XIII – TALK

## "INDICADORES DE ESTADOS EMOCIONAIS POSITIVOS EM OVINOS" IN I SIMPÓSIO EM BEM-ESTAR ANIMAL, PONTIFÍCIA UNIVERSIDADE CATÓLICA DO PARANÁ - PUCPR

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO PARANÁ

MEDICINA Veterinária

**I SIMPÓSIO EM BEM-ESTAR ANIMAL**

Declaramos que

**PRISCILA TAMIOSO**

Participou como palestrante no I Simpósio em Bem-estar Animal, realizado de 03 a 04 de junho de 2016, na PUCPR/Curitiba, organizado pela Comissão de Formatura de Medicina Veterinária 12-16 PUCPR.

**Claudia Turra Pimpão**
Coordenadora do Curso de Medicina Veterinária

PUCPR

MEDICINA VETERINÁRIA PUCPR

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**Flávia Borges Santos**
Presidente da Comissão de Formatura
MEDVET 12-16

## APPENDIX XIV – ABSTRACT

## "BEHAVIOURAL INDICATORS AND TEMPERATURE IN RAMS IN RELATION TO FEED ENVIRONMENTAL ENRICHMENT: PRELIMINARY RESULTS"

TAMIOSO, P.R.[1]; GONZALEZ, D.S.R.[1]; BERGSTEIN, T.G.[2]; MOLENTO, C.F.M.[1]
[1]Laboratório de Bem-estar Animal – LABEA, Universidade Federal do Paraná, Curitiba, Paraná, Brasil. priscillatamioso@gmail.com, carlamolento@ufpr.br
[2]Pós-graduação em Engenharia de Bioprocessos e Biotecnologia – PGEBB, Universidade Federal do Paraná, Curitiba, Paraná, Brasil.

Environmental enrichment (EE) is proposed as a way to promote positive experiences in captive animals. We aimed to study behaviour and temperature before, during and after EE through the offer of hay in a long tube-shaped device, with openings of different sizes, for 15 min. Eight Dorper rams (18±5 months old on average) were assessed. Behaviour was recorded for 3 min per period. Vocalization, body movements, tail wagging, ear postures and changes and half-closed eyes were assessed. We also recorded temperature at withers (Tw) with an infrared thermometer. Each animal was assessed once. Behaviour was analyzed through descriptive statistics and temperature with Friedman test for pairwise comparisons. All rams interacted with the device. The animals did not vocalize, moved for 18 (80/0) before, 1.5 (32/0) during and 7.5 (29/4) s after EE, and did not change ears frequently nor wagged tails. A higher duration of body moves before EE may indicate anticipation of a positive event. Moreover, the animals expressed passive ears for 128.5 (175/54) before (5/8), 179 (180/134) during and 136.5 (179/71) s after EE. An apparent higher proportion of passive ears during EE may be an indicator of a positive experience. Half-closed eyes were not observed throughout the study. Regarding Tw, there was no difference between periods ($p>0.05$), with medians before, during and after EE of 29.3 (34.2/25.1), 30.6 (33.3/26.8) and 29.55 (34.2/26.2)$^{o}$C, respectively. The results on ear postures and anticipatory movement suggest that the proposed EE device may elicit positive experiences in sheep and warrant further studies.

# APPENDIX XV – PAPER

# "EMOÇÕES, SENTIMENTOS E SUA RELEVÂNCIA PARA O BEM-ESTAR ANIMAL"

Priscilla Regina Tamioso, Vanessa Carli Bones, Daniel Santiago Rucinque Gonzalez, Carla Forte Maiolino Molento
Laboratório de Bem-estar Animal – LABEA, Universidade Federal do Paraná, Curitiba, Paraná, Brasil. Autora para correspondência: carlamolento@ufpr.br

A capacidade de sentir em animais é foco de discussão crescente no ambiente científico e também gera movimentos sociais cada vez mais fortes e frequentes. A importância de tal discussão pode ser justificada pelo fato de que, se os animais têm sentimentos e emoções, eles não são objetos, e consequentemente não devem ser tratados como coisas (DECLARAÇÃO DE CURITIBA, 2014). Embora haja reconhecimento de que os animais não são coisas, que há diferenças entre um cão e uma cadeira, as ações humanas revelam outra realidade. Animais são vendidos, usados em pesquisa, na produção de alimentos e em vários outros contextos. Sob o ponto de vista legislativo, a situação é também crítica, pois os animais são qualificados como coisas, bens de propriedade, salvo raras exceções. Logo parece importante olhar tal questão com atenção e buscar uma compreensão mais refinada sobre a senciência animal.

A senciência animal, entendida como um nível primário de consciência, pode ser resumida como a capacidade de sentir. O verbo sentir, segundo o dicionário Aurélio, é perceber por qualquer órgão dos sentidos, experimentar, pressentir, ter consciência de; notar, perceber, tomar consciência ou percepção do próprio estado ou condição, reconhecer-se (FERREIRA, 2005). Mesmo com uma gama de estudos sobre as formas de expressão de sentimentos e emoções em animais, não existe um consenso entre os pesquisadores no que tange a definições desses termos. Recentemente, MELLOR (2015), em artigo de revisão sobre a relação entre bem-estar animal e estados emocionais positivos, evidencia o grau de sobreposição dos termos "experiências subjetivas", "emoções", "sentimentos" e "estados afetivos", além da dificuldade em estabelecer distinções entre os mesmos. Diante do exposto, o objetivo deste texto é apresentar as bases do reconhecimento científico atual da senciência animal e as principais definições dos termos "emoção" e "sentimento", abordando a relevância de tais conceitos para o bem-estar animal.

## O reconhecimento da senciência animal

Evidências científicas indicam que seres humanos e animais compartilham substratos neurológicos que geram consciência (THE CAMBRIDGE DECLARATION ON CONSCIOUSNESS, 2012). Adicionalmente, REGAN (1983) lista na teoria cumulativa para o reconhecimento da senciência animal evidências neurológicas, comportamentais, farmacológicas, evolutivas e bom senso. O bom senso, aqui caracterizado por uma noção leiga de que os animais sentem, apresenta uma natureza interessante por sustentar a reversão do ônus da prova científica. Já pareceu óbvio que o planeta Terra fosse plano, quem pensasse o contrário deveria apresentar provas científicas irrefutáveis para convencer a sociedade. De forma semelhante sabe-se que os animais sentem, quem defende o contrário deve apresentar provas científicas irrefutáveis para apoiar sua argumentação.

Apesar das evidências cumulativas de consciência em diferentes espécies, há cientistas que parecem sentir-se desconfortáveis com o uso de termos como emoção ou sentimento em relação aos animais, uma vez que tal atribuição pode caracterizar antropomorfismo (PAUL *et al.*, 2005). Entretanto, a desconsideração da discussão baseada em menção ao antropomorfismo parece desqualificada, uma vez que somente se pode considerar antropomórfica a atribuição a outrem de característica exclusivamente humana (REGAN, 1983), o que no caso de emoções e sentimentos não foi demonstrado.

No Brasil, estudos acerca da avaliação de estados afetivos em animais têm recebido destaque desde a década de 60. O professor Cesar Ades, do Instituto de Psicologia da USP, apresentou relevante contribuição para a área do comportamento animal, sendo considerado um dos pais da etologia no país. Sua pesquisa envolveu temas diversos, incluindo percepção, motivação, aprendizagem, memória, comunicação e emoção, tanto em seres humanos quanto em animais. Segundo o pesquisador, a dificuldade em entender a mente de um animal é análoga à dificuldade de conhecer a qualidade do pensamento ou das emoções de um ser humano e, neste sentido, uma abordagem centrada na cognição e na consciência animal tem sido desenvolvida (ADES, 1997).

Embora haja diversos grupos em vários países com linhas de pesquisa envolvendo senciência animal, o conhecimento de estados afetivos nos animais ainda é incipiente. O fato de que eles não podem relatar verbalmente como se sentem parece explicar em parte a situação. O relato verbal tem limitações, pois em estudos com seres humanos observaram-se discrepâncias entre o que é relatado verbalmente e o que de fato é sentido (ZAMMUNER, 1996). Entretanto, a linguagem verbal permanece como um referencial importante para a compreensão dos sentimentos em seres humanos. De maneira similar, os animais se expressam por meio de diferentes tipos de comunicação. Exemplos de formas de comunicação em animais incluem variações de grunhidos dependendo do predador avistado por macacos; rugidos em veados machos durante as disputas por fêmeas, sendo que rugidos frequentes e intensos têm grande potencial para afugentar o oponente; penas do pescoço arrepiadas, cabeças erguidas e ataques indicativos de dominância em galinhas; cantos para a defesa de território em galos e outras aves; leitura e interpretação de sinais humanos em equinos e chimpanzés; danças de abelhas para indicar a localização de alimentos às companheiras, assim como respostas a testes de preferência e de esforço (DAWKINS, 1998), entre outras linhas de estudo. Pelos exemplos citados, é fácil notar que a ausência de linguagem verbal não significa ausência de comunicação efetiva. O estudo da comunicação animal pode revelar avanços importantes para o conhecimento de emoções e sentimentos em diversas situações.

Apesar das dificuldades, há indícios concretos da presença de emoções e sentimentos com algum grau de consciência em animais. Exemplos específicos incluem os sinais de medo frente a predadores em macacos, o sofrimento causado por injúrias resultantes de brigas entre machos de veados para disputa de fêmeas, a sensibilidade de perceber quando animais familiares estão doentes em ratos usados em laboratório (DAWKINS, 1998), a capacidade de encontrar alimentos, a construção de artefatos e ferramentas para facilitar a alimentação em primatas (GRIFFIN, 2001) e também a comunicação entre animais e seres humanos (DAWKINS, 1998; GRIFFIN, 2001).

Qual a diferença entre emoções e sentimentos?

Com frequência, os termos emoção e sentimento são empregados como sinônimos na literatura. Isto se caracteriza sobretudo quando autores utilizam a expressão "emotional feeling" (GRIFFIN, 2001; MENDL *et al.*, 2010; DOLAN, 2002). A variedade de conceitos propostos para a definição de emoções e sentimentos também aumenta a complexidade no estudo de tais termos. As tabelas 1 e 2 exemplificam a diversidade de tais definições, principalmente em relação a emoções. Por exemplo, KLEINGINNA & KLEINGINNA (1981) compilaram 92 definições de emoções e sentimentos a partir de diferentes autores, salientando que os termos são frequentemente confundidos.

Tabela 1. Definições do termo "emoções" com respectivos autores e páginas.

| Definição | Autor |
|---|---|
| Processos psiconeurais que são especialmente influentes no controle do vigor e padronização de ações no fluxo dinâmico de intercâmbios comportamentais intensos entre animais, assim como com determinados objetos durante circunstâncias que são especialmente importantes para a sobrevivência. | PANKSEPP (1998), p.48 |
| Sentimentos e pensamentos distintos, estados psicológicos e biológicos, e gama de tendências para agir. | GOLEMAN (2001), p.303 |
| Respostas afetivas intensas, mas curtas, a um evento e materializadas em mudanças corporais específicas. Além disso, são fenômenos multidimensionais que compreendem componentes cognitivo, neurofisiológico, de expressão motora ou comportamental, motivacional e sentimento subjetivo; sendo que os componentes motivacional e de sentimento subjetivo nos animais, por não possuírem linguagem verbal, podem ser medidos somente por meio dos outros componentes. | DÉSIRÉ *et al.* (2002), p.166 e 177 |
| Combinações de processos avaliatórios mentais, simples ou complexos, com respostas dispositivas a esses processos, em sua maioria dirigidas ao corpo propriamente dito, resultando em estados emocionais do corpo, mas também ao próprio cérebro (núcleos neurotransmissores no tronco cerebral), resultando em alterações mentais adicionais. | DAMÁSIO (2005), p.168 e 169 |
| Episódios de mudanças inter-relacionadas, sincronizadas nos estados de todos ou da maioria dos cinco subsistemas orgânicos em resposta à avaliação de um evento de estímulo externo ou interno como relevante para grandes preocupações do organismo. | SCHERER (2005), p.697 |
| Circuitos neurais (que são, pelo menos, parcialmente empregados), sistemas de respostas, e um estado/processo de sentimento que motiva e organiza a cognição e a ação. | IZARD (2010), p.367 |
| Programas de ação em grande parte desencadeados por estímulos externos (percebidos ou lembrados). Exemplos incluem aversão, medo, raiva, tristeza, alegria, vergonha, desprezo, orgulho, compaixão e admiração. | DAMÁSIO & CARVALHO (2013), p.145 |
| Respostas automáticas, intensas, rápidas, conscientes ou inconscientes, e impulsos neuronais que levam o organismo a promover uma ação do corpo. | FREITAS-MAGALHÃES (2013), p.34 |
| Componentes fisiologicamente descritíveis de um sentimento, caracterizados pela atividade elétrica e neuroquímica em determinadas regiões do cérebro, atividade do sistema nervoso autônomo, liberação hormonal e consequências periféricas, incluindo o comportamento. | BROOM (2014), glossário, p.xi e xii |

No que tange a emoções em animais, elas foram descritas já em 1872 por Charles Darwin, no livro "A expressão das emoções nos homens e nos animais" (The Expression of Emotions in Man and Animals) (DARWIN, 2000). Na referida obra o naturalista relacionou emoções como expressões faciais estereotípicas e posturas

corporais em contextos específicos. Além disso, Darwin expôs similaridades entre expressões em seres humanos e animais, indicando ligação evolutiva entre as espécies, e catalogou diferentes maneiras nas quais os animais expressam uma variedade de emoções, como medo e raiva. Uma estrutura teórica mais atual de emoções em animais foi proposta por MENDL *et al.* (2010), os quais descreveram o estudo de estados afetivos por meio de espaços bidimensionais: valência, variando de agradável (positiva) a desagradável (negativa), e excitação (do inglês, *arousal*), variando de alta a baixa.

Tabela 2. Definições do termo “sentimentos” com respectivos autores e páginas.

| Definição | Autor |
|---|---|
| Representações mentais de alterações fisiológicas que caracterizam e resultam do processamento de objetos ou estados que eliciam emoções. | DOLAN (2002), p.1193 |
| Processos de acompanhamento contínuo, experiências do que o corpo está fazendo enquanto pensamentos sobre conteúdos específicos continuam a desenrolar-se. | DAMÁSIO (2005), p.175 |
| Sentimentos afetivos são as experiências fenomênicas com valência que podem assumir formas e variedades desejáveis (positiva) ou indesejáveis (negativa). Além disso, o sentimento subjetivo é entendido como o componente afetivo das emoções. | PANKSEPP (2011), p.1791 e 1792 |
| Experiências mentais que acompanham os estados corporais. Os programas de ação (pulsões e emoções) podem suscitar sentimentos. Esta definição também exclui o uso de “sentimento”, no sentido de "pensar" ou "intuir". | DAMÁSIO & CARVALHO (2013), p.145 |
| Construtos cerebrais, envolvendo, pelo menos, consciência perceptiva, que é associada a um sistema de regulação da vida, são reconhecíveis pelo indivíduo quando se repetem e podem alterar o comportamento ou agir como um reforço na aprendizagem. | BROOM (2014), glossário, p.xi e xii |

A consideração dos conceitos evidencia a necessidade de cautela no seu uso e na sua aplicação em pesquisas que visem estudar emoções e sentimentos em animais, em função da variação e da sobreposição existentes. Segundo BROOM (2014), emoções e sentimentos são às vezes usados de forma intercambiável, sendo que os sentimentos são decorrentes do cérebro e envolvem processamentos sofisticados, ao passo que emoções podem ser descritas mais prontamente em termos fisiológicos; porém, isto não impede a existência de componentes cognitivos na expressão das emoções. Desta maneira, emoções, no sentido de atividades neurais nos centros emocionais do cérebro ou mudanças hormonais específicas, podem ocorrer sem a presença de sentimentos, sendo que os sentimentos envolvem necessariamente consciência (BROOM, 1998). Logo, parece haver certa convergência no entendimento de que sentimentos estão relacionados a experiências mentais, enquanto que emoções podem ser descritas em termos de reações corporais químicas, endócrinas e neurais.

Relevância para o bem-estar animal

De que forma o entendimento dos conceitos de emoções e sentimentos pode ser relevante para o bem-estar animal? De acordo com BROOM (1998), os sentimentos de um indivíduo, juntamente com sua fisiologia e seu comportamento, contribuem para que o animal possa lidar com o ambiente de forma mais adequada e manter o equilíbrio do seu organismo em diversas situações. O medo, por exemplo, ocorre quando o animal enfrenta uma situação de potencial perigo e sua

presença normalmente resulta em fuga, defesa ou redução da atividade comportamental e fisiológica para se proteger de um predador.

Além disso, emoções e sentimentos são parte integrante dos argumentos para definir o bem-estar animal. WEBSTER (2005) formulou três perguntas que podem auxiliar a definir o bem-estar animal: o animal vive no seu ambiente natural?; o animal está saudável e tem um crescimento normal?; o animal tem satisfações mentais, ou pelo menos, é livre de sofrimento mental?. O argumento que responde esta última pergunta pode ser caracterizado pelas emoções e pelos sentimentos predominantes. Assim, quando o animal apresenta emoções e sentimentos positivos, o seu grau de bem-estar será maior se comparado a outro que vivencia emoções e sentimentos negativos.

Pode-se concluir que a consideração das emoções e dos sentimentos é fundamental para a compreensão das consequências das formas de manutenção e do tratamento oferecido aos animais. Consequentemente, a consideração das emoções e dos sentimentos é essencial para propostas de melhoria da qualidade de vida dos animais. A partir de avanços na compreensão de sentimentos e emoções será possível um aumento no entendimento de como os animais percebem o mundo, do significado para eles dos problemas que enfrentam e dos benefícios que eventualmente podem desfrutar. Por meio de uma busca contínua desta compreensão será possível um trabalho cada vez mais eficaz pelo aumento do bem-estar dos animais.

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# APPENDIX XVI – LABEA NEWSLETTER

# "O TRABALHO DO LABEA/UFPR NO ESTUDO DE ESTADOS EMOCIONAIS EM ANIMAIS E PERCEPÇÃO DA SOCIEDADE EM RELAÇÃO À SENCIÊNCIA ANIMAL"

Laboratório de Bem-estar Animal da Universidade Federal do Paraná LABEA/UFPR
Boletim informativo número 4, Curitiba, 01 de dezembro de 2014
Priscilla Regina Tamioso, Bióloga, MSc em Ciências Veterinárias, Doutoranda pelo LABEA/UFPR, Curitiba-PR. E-mail: priscillatamioso@gmail.com
Carla Forte Maiolino Molento, Médica Veterinária, MSc, PhD, Professora de Bem-estar Animal e Coordenadora do LABEA/UFPR, Curitiba-PR. E-mail: carlamolento@ufpr.br

Nos últimos anos observou-se expansão na publicação de estudos científicos relacionados à senciência de diversas espécies, destacando-se os mamíferos. Segundo BROOM (2010) um ser senciente apresenta algum grau de consciência e/ou percepção e possui habilidade para avaliar as ações de outros em relação a si mesmo, bem como lembrar essas ações e consequências. Nota-se tendência, também, em publicações com foco na avaliação de experiências positivas nos animais (WEMELSFELDER & FARISH, 2004; YEATES & MAIN, 2008), sobretudo em espécies utilizadas para a produção de alimentos.

Neste contexto, o LABEA apresenta linha de pesquisa em construção na área de avaliação de estados emocionais. O mestrando Bruno Roberto Muller desenvolve projeto com o objetivo de investigar se unidades de ação da expressão facial, relacionadas à dor em outras espécies, também são ativadas em bovinos de corte submetidos à marcação a ferro quente. Resultados preliminares indicam que das 15 unidades de ação, ou expressões faciais específicas, avaliadas, cinco apresentaram associação significativa entre sua ativação e o estímulo doloroso: orelhas para trás ($P=0.0078$); narina dilatada ($P<0.0001$); abertura da boca ($P<0.0001$); e elevação medial ($P=0.0074$) e lateral da sobrancelha ($P<0.0001$). Além destas, a exposição da língua para fora também apresentou uma tendência de associação com o estímulo doloroso ($P=0.0625$) (Figura 1).


Figura 1. Unidades de ação narina dilatada (1), abertura da boca (2), elevação medial (3) e lateral (4) da sobrancelha no mesmo animal em momentos antes (A) e depois (B) da marcação a ferro quente. Créditos: Bruno R. Muller

Similarmente, a doutoranda Priscilla Regina Tamioso estuda indicadores comportamentais e fisiológicos de estados emocionais positivos em ovinos. Durante experimentos pilotos, ovinos de diferentes categorias foram submetidos a estímulos positivos, como oferta de alimento concentrado (ração), escovação e enriquecimento ambiental (Figura 2).

Figura 2. Estímulos positivos aos quais os ovinos foram submetidos: a) Oferta de alimento concentrado, para carneiros Dorper b) Escovação em ovelha vazia Dorper c) Enriquecimento ambiental, em lote de cordeiros desmamados mestiços. Créditos: Priscilla R. Tamioso

O LABEA atua também como peça importante no desenvolvimento de pesquisas acerca da percepção da população em relação à senciência animal. Tais pesquisas corroboram uma tendência internacional de crescente interesse em relação a questões éticas e de qualidade de vida animal por parte de diferentes segmentos da sociedade, sendo que esta preocupação transfere-se às escolhas dos consumidores em relação a produtos com maiores índices de bem- estar e menor grau de sofrimento (MAYFIELD *et al.*, 2007). Atualmente, mestrandos e doutorados do LABEA distribuem questionários online à população paranaense a respeito da percepção humana sobre emoções em animais e questões de bem-estar (link da pesquisa: http://www.survio.com/survey/d/beasenciencia) e bem-estar, abate humanitário e senciência em peixes (link da pesquisa: http://www.survio.com/survey/d/abatepeixesvf).

No que tange à promoção de eventos sobre o tema senciência animal, vale a pena ressaltar também que, em 2012, foi promovido o IV Encontro LABEA: Consciência e Cognição Animal – Uma homenagem a César Ades e II Congresso Internacional da AMVEBBEA, o qual tratou sobre a consciência e capacidade cognitiva de animais de diversas espécies, por meio de palestras e discussões. Adicionalmente, em agosto/2014, durante o III Congresso Brasileiro de Bioética e Bem-estar Animal, em Curitiba/PR, foi produzida a Declaração de Curitiba, a qual justifica que animais não devem ser tratados como coisas, uma vez que são seres sencientes.

A avaliação de estados emocionais em animais de produção é uma linha de pesquisa em suas fases iniciais desenvolvimento, sobretudo em países europeus, representando o estado da arte em bem-estar animal. O LABEA espera contribuir com estudos com o estabelecimento da área em território nacional e avançar no conhecimento de indicadores de estados emocionais, com potencial de subsidiar estudos similares para outras espécies. Propõe-se, também, expandir estudos a respeito da percepção humana da presença de senciência em diferentes espécies animais.

Bibliografia consultada

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# ANNEX I – SCIENCE WITHOUT BORDERS GRANT

CNPq - Resultado de Julgamento [206931/2014-0] - Doutorado Sanduíche - SWE Viagem x

**Cnpq** <dabs@cnpq.br> 01/12/2014

para priscilla_regi., mim, coagr, cgapb

Nome: Priscilla Regina Tamioso
Processo: 206931/2014-0
Modalidade: Doutorado Sandwich no Exterior - SWE
Instituição: Centre Clermont-Ferrand-Theix-Lyon - França
Chamada: Doutorado Sanduíche - SWE

Prezado (a) Senhor(a),

Comunicamos que, com base na recomendação do Comitê de Medicina Veterinária, a Diretoria do CNPq aprovou a concessão da bolsa discriminada acima pelo período de 4 meses.

Para a implementação da bolsa é necessário preencher e realizar o envio eletrônico do Formulário de Dados Complementares de Bolsa no Exterior que se encontra disponível através da chave de acesso abaixo:

http://efomento.cnpq.br/efomento/termo?token=StP81808W6577055510880935093904 2

Após o processamento do formulário de Dados Complementares, o CNPq lhe enviará mensagem eletrônica contendo uma chave de acesso para emissão do Termo de Concessão. O CNPq realizará o pagamento dos valores relativos à passagem aérea e auxílio instalação, após a assinatura eletrônica do Termo de Concessão pelo seu representante legal e a respectiva publicação no Diário Oficial da União.

O atendimento a proponentes com dúvidas ou dificuldades no preenchimento do Formulário de Dados Complementares, se dará através do telefone 0800.61.9697, de segunda a sexta-feira, no horário de 8h30 às 18h30, ou através do e-mail atendimento@cnpq.br. No caso de envio de email, solicitamos que sejam incluídos na mensagem nome completo, CPF e número do processo.

Atenciosamente,

Marcelo Marcos Morales
Diretor de Ciências Agrárias, Biológicas e da Saúde
dabs@cnpq.br

# ANNEX II – ANIMAL ETHICS COMMITTEE APPROVAL

**Universidade Federal do Paraná**
**Setor de Ciências Agrárias**
**Comissão de Ética no Uso de Animais – CEUA SCA**

CERTIFICADO

Certificamos que o protocolo no. 025/2014, referente ao projeto "Indicadores de estados emocionais positivos em ovinos", sob a responsabilidade de Priscilla Regina Tamioso, na forma em que foi apresentado (uso de 180 ovinos), foi aprovado pela Comissão de Ética no Uso de Animais do Setor de Ciências Agrárias, em reunião realizada dia 31 de julho de 2014.

CERTIFICATE

We certify that the protocol number 025/2014, regarding the project "Indicators of positive emotional states in sheep", under Priscilla Regina Tamioso's supervision, in the terms it was presented (use of 180 sheep), was approved by the Animal Use Ethics Committee of the Agricultural Sciences Campus of the Universidade Federal do Paraná (Federal University of Paraná, Brazil) during session on July 31st, 2014.

Curitiba, 31 de Julho de 2014.

Ricardo Guilherme D'Otaviano de Castro Vilani
Presidente

Ananda Portella Félix
Vice-Presidente

Comissão de Ética no Uso de Animais
Setor de Ciências Agrárias
Universidade Federal do Paraná.

# ANNEX III – HUMAN RESEARCH ETHICS COMMITTEE APPROVAL

UNIVERSIDADE FEDERAL DO PARANÁ - SETOR DE CIÊNCIAS DA SAÚDE/ SCS -

Plataforma Brasil

## PARECER CONSUBSTANCIADO DO CEP

**DADOS DO PROJETO DE PESQUISA**

**Título da Pesquisa:** PERCEPÇÃO E ATITUDES HUMANAS SOBRE A SENCIÊNCIA ANIMAL E QUESTÕES RELACIONADAS AO BEM-ESTAR ANIMAL

**Pesquisador:** Carla Forte Maiolino Molento

**Área Temática:**

**Versão:** 3

**CAAE:** 34820114.0.0000.0102

**Instituição Proponente:** Programa de Pós-graduação em Ciências Veterinárias

**Patrocinador Principal:** Financiamento Próprio

**DADOS DO PARECER**

**Número do Parecer:** 814.835

**Data da Relatoria:** 26/09/2014

**Apresentação do Projeto:**

PERCEPÇÃO E ATITUDES HUMANAS SOBRE A SENCIÊNCIA ANIMAL E QUESTÕES RELACIONADAS AO BEM-ESTAR ANIMAL

Carla Forte Maiolino Molento

Trata-se de projeto de pesquisa amplo, linha de pesquisa da Prof. Dra. Carla Forte Maiolino, que visa avaliar a percepção e atitudes humanas sobre a senciência animal e questões relacionadas ao bem estar animal.

**Objetivo da Pesquisa:**

Geral: Estudar a percepção humana a respeito da capacidade de sentir (senciência) em diferentes espécies de animais e questões de bem-estar.

Específicos:

- Estudar a percepção de biólogos, médicos veterinários, zootecnistas, consumidores e produtores de ovinos no estado do Paraná em relação à presença de senciência e estados afetivos nos animais desta espécie,

**Endereço:** Rua Padre Camargo, 280
**Bairro:** 2ª andar **CEP:** 80.060-240
**UF:** PR **Município:** CURITIBA
**Telefone:** (41)3360-7259 **E-mail:** cometica.saude@ufpr.br

aplicando-se um questionário e comparando à população europeia;- Estudar a percepção de produtores em relação a diferentes práticas de rotina realizadas em animais de
produção;
- Avaliar atitudes dos produtores para a realização de diferentes práticas de manejo e/ou rotina realizadas em animais de produção;
- Conhecer as percepções da população em questões relacionadas ao bem-estar e abate de peixes no Brasil e na Colômbia;
- Avaliar as atitudes relacionadas ao sofrimento e maus tratos em diferentes espécies.
- Realizar pesquisa com produtores de ovinos associados à Associação Paranaense de Criadores de Ovinos (OVINOPAR) e/ou à Castrolanda para determinar a motivação para realização da caudectomia;

**Avaliação dos Riscos e Benefícios:**
De acordo com os pesquisadores a aplicação de questionários para avaliar a percepção dos respondentes pode ser considerada como de baixo risco. Os tipos de risco mais prováveis são a possibilidade de exposição de informações demográficas (idade, sexo, renda, grau de escolaridade), as quais serão confidenciais, anônimas, e constrangimento pela possibilidade de provocar experiências ou recordações de situações vividas que causaram algum tipo de sofrimento psíquico. Além disso, o respondente pode acreditar que está correndo risco de ser julgado, entretanto será disponibilizado espaço apropriado para que sejam realizados comentários referentes à pesquisa, com o objetivo de minimizar tal efeito.

**Comentários e Considerações sobre a Pesquisa:**
A maior força motriz para a melhoria do bem-estar animal é a opinião pública. Há grande demanda do público por melhorias no bem-estar animal dos animais. Essa demanda se reflete nas atividades de várias organizações sem ânimo de lucro e através dos meios de comunicação social e, finalmente, a nível político, através de uma melhor legislação. Consequentemente a melhoria de vida dos
animais pode acarretar em bem-estar humano a partir do consumo de produtos provenientes de

animais com qualidade de vida adequada e condições de manutenção eticamente aceitáveis.

**Considerações sobre os Termos de apresentação obrigatória:**
Todos os termos obrigatórios foram anexados.

**Recomendações:**
Solicitamos que sejam apresentados a este CEP, relatórios semestrais e final, sobre o andamento da pesquisa, bem como informações relativas às modificações do protocolo, cancelamento, encerramento e destino dos conhecimentos obtidos, através da Plataforma Brasil - no modo: NOTIFICAÇÃO. Demais alterações e prorrogação de prazo devem ser enviadas no modo EMENDA. Lembrando que o cronograma de execução da pesquisa deve ser atualizado no sistema Plataforma Brasil antes de enviar solicitação de prorrogação de prazo.

**Conclusões ou Pendências e Lista de Inadequações:**
As pendências foram atendidas.
É obrigatório retirar na secretaria do CEP/SD uma cópia do Termo de Consentimento Livre e Esclarecido com carimbo onde constará data de aprovação por este CEP/SD, sendo este modelo reproduzido para aplicar junto ao participante da pesquisa.

O TCLE deverá conter duas vias, uma ficará com o pesquisador e uma cópia ficará com o participante da pesquisa (Carta Circular nº. 003/2011CONEP/CNS)

**Situação do Parecer:**
Aprovado

**Necessita Apreciação da CONEP:**
Não

**Considerações Finais a critério do CEP:**

UNIVERSIDADE FEDERAL DO PARANÁ - SETOR DE CIÊNCIAS DA SAÚDE/ SCS -

Continuação do Parecer: 814.835

CURITIBA, 01 de Outubro de 2014

**Assinado por:**
**IDA CRISTINA GUBERT**
**(Coordenador)**

**Endereço:** Rua Padre Camargo, 280
**Bairro:** 2ª andar **CEP:** 80.060-240
**UF:** PR **Município:** CURITIBA
**Telefone:** (41)3360-7259 **E-mail:** cometica.saude@ufpr.br