UNIVERSIDADE FEDERAL DO PARANÁ

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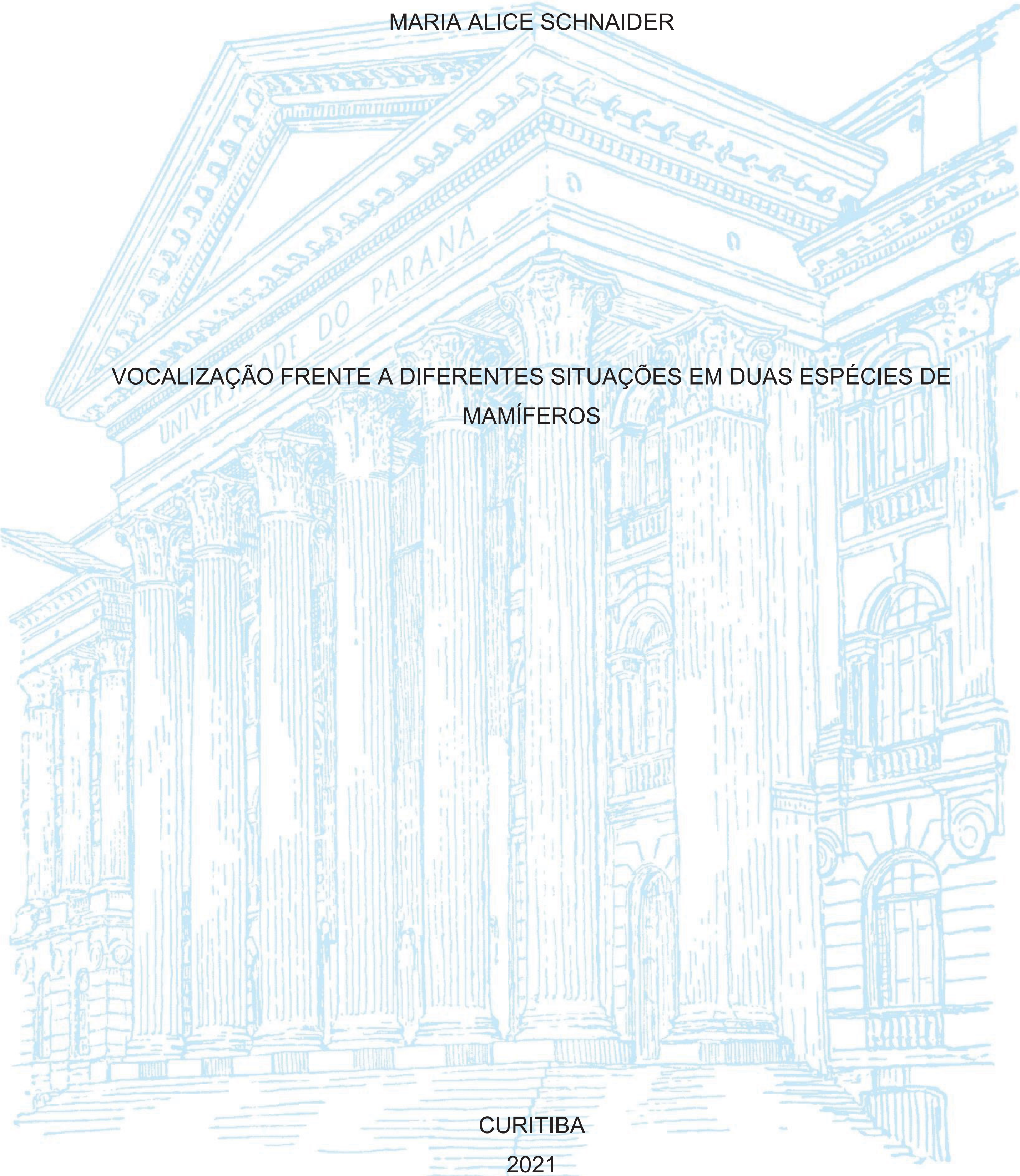

VOCALIZAÇÃO FRENTE A DIFERENTES SITUAÇÕES EM DUAS ESPÉCIES DE MAMÍFEROS

CURITIBA

2021

MARIA ALICE SCHNAIDER

VOCALIZAÇÃO FRENTE A DIFERENTES SITUAÇÕES EM DUAS ESPÉCIES DE MAMÍFEROS

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

Orientadora: Profa. Dra. Carla Forte Maiolino Molento
Coorientadora: Profa. Dra. Adelaide Hercília Pescatori Silva

CURITIBA

2021

Schnaider, Maria Alice
Vocalização frente a diferentes situações em duas espécies de mamíferos. / Maria Alice Schnaider. - Curitiba, 2021.

Tese (Doutorado) - Universidade Federal do Paraná. Setor de Ciências Agrárias, Programa de Pós-Graduação em Ciências Veterinárias.
Orientação: Carla Forte Maiolino Molento.
Coorientação: Adelaide Hercília Pescatori Silva
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1. Animais - Aspectos sociais. 2. Avaliação emocional. 3. Animais - Sons. 4. Animais - Comportamento. I. Molento, Carla Forte Maiolino. II. Silva, Adelaide Hercília Pescatori. III. Título. IV. Universidade Federal do Paraná.

Sistema de Bibliotecas/UFPR
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PROGRAMA DE PÓS-GRADUAÇÃO CIÊNCIAS
VETERINÁRIAS - 40001016023P3

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIAS VETERINÁRIAS da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **MARIA ALICE SCHNAIDER** intitulada: **VOCALIZAÇÃO FRENTE A DIFERENTES SITUAÇÕES EM DUAS ESPÉCIES DE MAMÍFEROS**, sob orientação da Profa. Dra. CARLA FORTE MAIOLINO MOLENTO, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.
A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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Aos meus pais e à minha filha Maria Fernanda.

AGRADECIMENTOS

A Deus, por me abençoar e me permitir realizar mais este sonho.

À minha filha Preciosa, Maria Fernanda, que é a minha força diária, que com o seu sorriso coloria os dias cinzentos e difíceis desta caminhada, que tem o abraço mais doce com o poder de me reconstruir e carregar as minhas energias, que enche de amor e esperança o meu coração, que ilumina os meus passos e dá sentido à minha vida, que é o meu maior e mais perfeito sonho realizado. Obrigada por ser MINHA, por ser a certeza que Deus existe e que é bondoso demais comigo. Nanda, filha amada, eu sempre farei de tudo para que você tenha orgulho e admiração pela sua mamãe e para que eu seja um bom exemplo para você. Você é o melhor presente que o Papai do Céu me deu e o motivo da minha felicidade, é absolutamente tudo para mim. Eu te amo infinitamente.

Aos meus pais, Sr. Schnaider e Sra. Maria do Carmo, pelo cuidado e proteção, por serem o meu porto seguro, meu esteio e sustentação, por serem os melhores pais do mundo, por me apoiarem em todas as minhas decisões, por me darem colo nas adversidades e me fazerem acreditar que tudo é possível, por serem meu abrigo em cada final de dia e pela benção de todas as despedidas: que Deus te abençoe, minha filha! E Deus abençoou, graças às suas preces. Gratidão eterna a vocês, esta conquista é nossa!

À minha mãe, que é a minha maior incentivadora, que sempre defendeu o aperfeiçoamento e o progresso da minha carreira profissional. Com certeza, a responsável por eu ter idealizado o doutorado. Por lutar pelo meu sucesso e das minhas irmãs, por nunca medir esforços para nos dar condições de estudo. Pelo amor incondicional, por ser o equilíbrio da nossa família, uma mãe e avó tão prestimosa que está sempre disponível para nos socorrer. Por ser perseverante e por ter se doado a vida inteira por todos nós. Obrigada por ser minha inspiração, por ser essa mulher espetacular que eu tanto amo e admiro.

Ao meu pai, por ser o meu melhor amigo, o meu primeiro grande amor, o meu xodó, a minha alma gêmea, minha estrela guia, meu mestre e professor. Obrigada por ser meu maior exemplo e influência, por me ensinar a amar a vida no campo e me dar a oportunidade de seguir os seus passos como pecuarista. Por me ajudar por anos com o projeto piloto e com a execução do projeto real a partir do estudo das suas vacas e bezerros. Pela paciência infinita durante as estações de parição do seu rebanho enquanto eu ocupei seus funcionários e o seu local de trabalho na fazenda com minhas pranchetas, tripés, câmeras e demais equipamentos. Obrigada por ser minha luz e amor maior.

Ao meu marido, Pedro, por abraçar os meus sonhos e junto a mim realizá-los, por ser meu cúmplice e companheiro de todas as horas, por ter me auxiliado na coleta dos dados do projeto e por ter cuidado com tanto amor, carinho e zelo da nossa filha enquanto eu estava ocupada com o doutorado. Pelos mates que me mantinham acordada escrevendo por horas, pelas pipocas doces e todas as delícias que cozinhava para me agradar, por fazer a Nanda dormir e voltar para me fazer companhia e mexer no meu cabelo enquanto eu lia infinitos artigos durante a madrugada. E claro, por ter tanta compreensão, por estar comigo abrindo mão dos finais de semana e datas festivas ao longo desses anos e por hoje estarmos unidos vibrando por mais esta vitória.

Às minhas irmãs, cunhados e sobrinhos que andaram de mãos dadas comigo e me acompanharam em todos os desafios que eu me propus a passar, que me deram força em todas as vezes que eu me sentia exausta, que me esperavam com chocolate, churrasco e todas as minhas comidas preferidas, que ficavam horas ao telefone me acalmando e me incentivando a prosseguir, que foram e são amor, afago e acalento em minha vida.

À minha orientadora, Profa. Dra. Carla Forte Maiolino Molento, por acreditar em mim e me permitir fazer parte da sua equipe maravilhosa, por me encorajar a sempre seguir em frente, por me ensinar sobre Bem-estar animal, por compartilhar seus valiosos conhecimentos comigo, me ajudar tanto durante toda essa jornada e tornar possível a concretização deste sonho.

À minha coorientadora, Profa. Dra. Adelaide Hercília Pescatori Silva, por ter sido meu ombro amigo em todos os momentos do doutorado, por me apresentar seus gatinhos que foram a razão do meu encantamento por vocalização felina. Obrigada por me ensinar sobre análise vocal e por me dar a honra de conviver com a senhora, uma pessoa com o coração generoso e solidário.

Ao professor Dr. César Augusto Taconeli, pela análise estatística do nosso trabalho e pela paciência ao nos explicar sobre cada método e resultado.

Às minhas amigas do LABEA, Vanessa, Ana Paula, Elaine, Marina, Juliana e Roberta, pela irmandade, cumplicidade e por tudo que vivemos juntas, tantas lembranças que ficarão guardadas em minhas melhores memórias e no lugar mais especial do meu coração. Obrigada, obrigada e obrigada.

À minha amiga Ludmylla, por mesmo de longe se fazer tão presente, pela amizade e lealdade nesses 17 anos, pelo companheirismo, por me motivar e torcer pela minha felicidade.

À minha amiga Marina, por ter sido uma benção de Deus em minha vida, pelas palavras que serviam como bálsamo para aliviar os momentos de tensão, por escutar os meus desabafos e me fazer ver o lado bom de cada situação, pelos conselhos e por toda a ajuda na materialização desta tese.

Aos tutores dos gatinhos que contribuíram com o estudo, em especial à professora Adelaide que me acolheu em sua casa e em sua família felina.

Aos animais que participaram do nosso projeto e cooperaram de forma brilhante para que pudéssemos estudar sobre suas vocalizações.

À Gislaine, da secretaria do Programa de Pós-graduação em Ciências Veterinárias, pela disponibilidade e benevolência, pela atenção oferecida e assistência prestada.

Aos professores Rafael e Júlia, pela colaboração durante o doutorado.

À Universidade Federal do Paraná, por possibilitar e viabilizar a pesquisa e o desenvolvimento da ciência.

À CAPES pelo apoio financeiro.

À Fazenda Nova Esperança, lugar em que me criei e que foi responsável pela minha formação pessoal e profissional, que me inspirou a ser Médica Veterinária,

Mestre em Ciência Animal e agora, Doutora em Ciências Veterinárias, que possibilitou a realização não somente deste sonho, mas de tantos outros. Que é a minha Querência e refúgio, o lugar para onde eu volto ao final de cada batalha e encontro a minha paz.

A todos que estiveram comigo nesta trajetória, obrigada de todo o meu coração.

A sua benção meu Pai, a sua benção minha Mãe.

RESUMO

O bem-estar animal pode ser medido usando uma combinação dos indicadores comportamental, fisiológico e neurológico. A avaliação do bem-estar animal deve ser prática, válida, repetível e robusta para uma abordagem de sucesso. Neste sentido, as vocalizações são ferramentas não invasivas com potencial para auxiliar na avaliação do bem-estar animal. Parâmetros acústicos como frequência fundamental, duração e taxa de vocalização podem ser associados a sinais de comportamento para fornecer informações sobre fisiologia, estado afetivo, atributos físicos do emissor e podem modular as emoções dos receptores. Assim, este trabalho teve como objetivo aprimorar o conhecimento a respeito da vocalização como expressão de emoções, comparando as características vocais e comportamentais apresentadas por gatos e bovinos de corte em diferentes situações. Esta tese está organizada em cinco capítulos: (1) Apresentação; (2) Vocalização do gato em situações aversivas e agradáveis; (3) Vocalização como indicador de valência emocional: o caso da separação e reencontro vaca-bezerro; (4) Vocalização e outros comportamentos que indicam dor em bezerros durante o procedimento de marcação de orelha; e (5) Considerações finais. O segundo capítulo compara as respostas vocais e comportamentais dos gatos domésticos durante uma situação aversiva e uma agradável. Além dos parâmetros vocais encontrados, houve correlação dos indicadores vocais, sexo e idade. Ainda, sugere-se uma relação entre o padrão da pelagem e a vocalização, uma vez que gatos com pelagem sólida apresentaram frequência fundamental mais alta que os outros. Em seguida, o estudo sobre as vocalizações e sinais comportamentais de pares de vaca e bezerro em situações emocionalmente negativas e positivas evidenciou alta frequência fundamental, chamadas vocais mais longas e mudanças comportamentais na separação vaca-bezerro. Ademais, o quarto artigo relata os parâmetros vocais e sinais comportamentais expressos por bezerros de corte submetidos ao procedimento de marcação auricular para identificação, que é um procedimento obrigatório para bezerros de corte em Santa Catarina, Sul do Brasil. Sugere-se que a marcação auricular é um procedimento doloroso devido ao aumento das vocalizações e movimentos corporais. De maneira geral, esta tese contribui para o avanço da compreensão sobre a associação entre a vocalização dos animais e os sinais de comportamento para melhorar o bem-estar animal. Assim, as vocalizações parecem ser uma ferramenta eficiente e viável para avaliar o bem-estar animal.

Palavras-chave: 1.Análise acústica; 2.Avaliação de emoções; 3.Bem-estar animal; 4.Comunicação vocal; 5.Parâmetros acústicos; 6.Sinais comportamentais.

ABSTRACT

Animal welfare can be measured using a combination of behavioural, physiological and neurological indicators . Animal welfare assessment should be practical, valid, repeatable and robust for success approach. In this regard, vocalizations are non-invasive tools with potential for assist animal welfare assessment. Acoustic parameters such as fundamental frequency, duration and vocalization rate can be associate to behaviour signals to provide information about physiology, affective state, physical attributes of the caller, and they can modulate the emotions of the receivers. Therefore, this work aimed to improve the knowledge regarding vocalization as an expression of emotions, comparing the vocal and behavioural characteristics exhibited by cats and beef cattle in different situations. This thesis is organized into five chapters: (1) Presentation; (2) Cat vocalization in aversive and pleasant situations; (3) Vocalization as an indicator of emotional valence: the case of cow-calf separation and reunion; (4) Vocalization and other behaviors indicating pain in beef calves during the ear tagging procedure; and (5) Final considerations. The second chapter compares the vocal and behavioural responses of domestic cats during an aversive and a pleasant situation. Besides the vocal parameters founded, there was a correlation of vocal indicators, sex and age. Also, it suggested a relationship between coat pattern and vocalization, as solid-coloured coated cats presented higher fundamental frequency than others. Then, the study regarding the vocalizations and behavioural signals by cow-calf pairs in emotionally negative and positive situations evidenced high fundamental frequency, longer vocal calls and behavioural changes in the cow-calf separation. Then, the fourth chapter reports vocal parameters and behaviour signals expressed by beef calves submitted to ear tagging procedure for identification, which is a mandatory procedure for beef calves in Santa Catarina, South Brazil. It suggested that ear tagging is a painful procedure due to the increase in vocalizations and body movements. Overall, this thesis contributes to advance the comprehension on the association between animal vocalization and behaviour signals to improve animal welfare. Thus, vocalizations seem to be an efficient and feasible tool to assess animal welfare.

Keywords: 1.Acoustic analysis; 2.Acoustic parameters; 3.Animal welfare; 4.Behavioral signs; 5.Emotion assessment; 6.Vocal communication

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1 PRESENTATION

Animal welfare is such an important concept in today's society yet it is difficult for animal scientists, producers, veterinarians and consumers to adequately and completely define (EDWARDS-CALLAWAY, 2015). Welfare has to be defined in such a way that it can be readily related to other concepts such as: needs, freedoms, happiness, coping, control, predictability, feelings, suffering, pain, anxiety, fear, boredom, stress and health (BROOM E MOLENTO, 2004). Thus, one of the main concepts used to define animal welfare is the state of an individual in coping with its environment (BROOM, 1991). In this sense, animal welfare can be measured using a combination of behavioural, physiological and neurological status (WILLIAMS, 2009). Routinely, a key method of animal welfare assessment is the Five Freedoms, written by the Brambell Committee in 1965, which includes: freedom from thirst, hunger and malnutrition; freedom from discomfort; freedom from pain, injury or disease; freedom to express most normal behaviour; and freedom from fear and distress. According to these principles, several protocols have been originated, such as the Welfare Quality, with specifications for many species.

Animal welfare assessment should be practical, valid (providing real information of welfare), repeatable and robust (not influenced by the weather, etc) for success approach (BUTTERWORTH et al., 2018). Behaviour has a major role in assess the current state of an animal, with crucial importance for determining what animals want, which includes observations of vocalizations (DAWKINS, 2004). It is possible to judge acoustically uttered current needs and impaired welfare by non-invasive, continuous monitoring (MANTEUFFEL et al., 2004). Vocal calls can provide conspecific with meaningful information about the physiology, affective state, and physical attributes of the caller (BLANK, 2020). Also, vocalizations convey a degree of interest attached to the stimulus and level of excitement (BLANK, 2020) and they can modulate emotions of the receivers (MANTEUFFEL et al., 2004; TURESSON e GHAZANFAR, 2009). Therefore, the analysis of vocalization can improve animal welfare due to its capability to assess stress in common procedures (GRANDIN, 2020).

Vocal calls have been evaluated from different species for a variety of situations. SUN et al. (2020) investigated the patterns and causes of geographical variation in acoustic signals and reported for the first time that bats can discriminate geographical variation in social calls. In this sense, vocalizations can provide information of habitat acoustics, which are used to induce group movements, defend territory or attack (TURESSON e GHAZANFAR, 2009). Also, regarding vocalizations in an emotional context, studies combine behaviour and physiological measures with the analysis of acoustic parameters to provide evidences for positive and negative valences. For instance, BACIADONNA et al. (2019) claimed that goats may discriminate emotions conveyed in calls emitted by conspecifics; FRIEL et al. (2019) and VILLAIN et al. (2020) explored the vocal expressions of pigs exposed to emotionally positive and negative situations, such as an enriched environment or pseudo-social events like the arrival of conspecifics to the pen; BURGDORF et al. (2008) studied the ultrasonic responses of rats during mating, play and aggression; and FONTANA et al. (2016) identified the vocal patterns in chickens regarding isolation and according to their age. Thus, even though distinct species present specific vocal types, it is notable that vocalization is an important behavior that can be used to distinguish animal emotional valence regarding aversive or pleasant situations.

The second chapter of this thesis is an analysis of vocal and behavioural responses of domestic cats during an aversive and a pleasant situation. According to the FEDERAL COUNCIL OF VETERINARY MEDICINE (2014), the population of cats in Brazil increases 8% per year. Thus, it is essential for tutors, veterinarians and other people responsible for these animals, such as shelter caretakers, the recognition of cat welfare by the evaluation of their vocalizations. In light of the relationship of humans and cats as companion animals, it is important to consider their interactions, such as cat responses to some humans' communicative cues (PRATO-PREVIDE et al., 2020). Also, even though cats exhibit a large repertoire of sounds (NTALAMPIRAS et al., 2019) the experiment reported in this chapter evaluated vocal acoustic parameters, such as fundamental frequency or pitch, duration and vocalization rates, as well as behaviour signals of cats exposure to two different contexts that are common in domestic cats' lifetime: car transportation within a box and offering a snack. This chapter was submitted to the Journal of Veterinary Behavior (ANNEX I). Additional data from this experiment that measured emitted vocalizations other than meowing was published in the journal Animals (ANNEX II).

Then, the third and fourth chapters are related to vocalizations of beef cattle. In this sense, animal welfare is now perceived as an important component of beef cattle management, and one that can have a positive impact on animal and human health (NALON et al., 2021). In the increasing pressure to intensify cattle production, people often ignore the fact that the unit in the factory production system is a higher mammal, with complex mental and physical needs (PHILLIPS, 2008). Cattle of all ages, both in traditional and modern housing systems, and under intensive and extensive management, suffer from a variety of diseases as well as various forms of physical injury as a result of poor housing or management (RUSHEN et al., 2007). Poor production and handling practices continue to persist that are detrimental to animal welfare (GRANDIN, 2018).

The third chapter is an analysis of vocalization and behavioural signs by cow-calf pairs in emotionally negative and positive situations. Vocalizations play an important role in eliciting a mother's responses and directing neonatal activities, and cow-calf separation can be stressful to both (BLANK, 2020). Therefore, the experiment consisted in two controversial phases: the separation of dam and offspring and their reunion. Vocal calls parameters and behaviour signals were evaluated to assess cattle emotional valences in both situations. Data from this chapter was submitted to the Journal of Veterinary Behavior (ANNEX III). Then, chapter four explored the pains signals expressed by beef calves submitted to ear tagging procedure for identification. Likewise, vocalization parameters were linked with behaviour signals to estimate calves discomfort in this standard handling procedure. This chapter was also submitted to the Journal of Veterinary Behavior (ANNEX IV).

In addition to this project, the author contributed with five articles during the postgraduate period. First, a paper that discuss the concept of compassionate conservation and its application in practice by addressing three relevant wildlife conservation issues, published in the journal Archives of Veterinary Science, in 2017 (ANNEX V). Also, a paper regarding the development and refinement of three animal-based broiler chicken welfare indicators (ANNEX VI) published in the journal Animal Welfare in 2018. Besides that, she participated as co-author to three articles entitled: a) “Attitude of Brazilian consumers on animal welfare”, with the objectives to study and identify consumer attitudes regarding animal welfare, possible advantages of food consumption from better welfare practices, and factors that may influence purchasing decisions; b) “Perception of beef cattle producers in the state of Paraná regarding animal identification by hot iron branding”, that reported the opinion of cattle producers in the State of Paraná, Brazil, indicating recognition of animal sentience and their ability to experience pain; and c) “Consequences of artificial selection to animal welfare”, which presented companion and farm animals' welfare impacts due to artificial selection. The last three papers were published in an especial edition of animal welfare and behaviour of the journal Revista Acadêmica: Ciência Animal, in 2018 (ANNEX VII to IV).

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2 CAT VOCALIZATION IN AVERSIVE AND PLEASANT SITUATIONS

RESUMO

A vocalização pode transmitir informações do animal emissor, incluindo seu estado emocional. Este estudo teve como objetivo comparar as respostas vocais e comportamentais de gatos domésticos durante uma situação aversiva e uma agradável. Participaram do estudo 74 gatos (29 machos e 45 fêmeas) da cidade de Curitiba, Sul do Brasil; 68 (26 machos e 42 fêmeas) foram divididos em dois tratamentos: situação aversiva (AS), com o evento de transporte de carro dentro de uma caixa, ou situação prazerosa (PS), quando lhes foi oferecido petisco. Os demais animais (três machos e três fêmeas) participaram das duas situações. Observações no local, vídeos e chamadas vocais foram registradas e foram observadas diferenças entre os parâmetros vocais e sinais comportamentais em cada cenário. Os gatos do PS apresentaram maior frequência fundamental (10,1%), menor tessitura (33,9%) e o dobro da taxa de movimentação de cabeça em relação ao AS, demonstrando respostas específicas em cada situação. Para a duração de chamada, houve interação significativa entre o tratamento e sexo. Além disso, houve diferenças entre sexo, idade e cor da pelagem. Fêmeas e filhotes apresentam frequências fundamentais mais altas, podendo ser devido a características anatômicas. Os resultados também sugerem uma relação entre a cor da pelagem e o temperamento, uma vez que os gatos com pelagem de cor sólida apresentaram frequência fundamental maior do que outras cores de pelagem. Em geral, os parâmetros vocais e os sinais comportamentais parecem ser indicadores úteis para estudar as emoções dos gatos em diferentes situações; mais estudos são necessários para compreender as sutilezas da vocalização do gato em relação ao sexo, idade e cor da pelagem.

Palavras-chave: 1.Análise acústica; 2.Avaliação de emoções; 3.Bem-estar animal; 4.Comunicação vocal; 5.Parâmetros acústicos; 6.Sinais comportamentais.

ABSTRACT

Vocalization may transmit information from the emitting animal, including his or her emotional state. This study aimed to compare the vocal and behavioral responses of domestic cats during an aversive and a pleasant situation. A total of 74 cats (29 males and 45 females) in the city of Curitiba, Southern Brazil, participated in the study; 68 (26 males and 42 females) were divided into two treatments: an aversive situation (AS), with a car transport event within a box, or a pleasant situation (PS), when they were offered a snack. The other animals (three males and three females) participated in both situations. Local observations, videos and vocal calls were registered and differences were observed between vocal parameters and behavioral signals in each scenario. Cats in the PS presented higher fundamental frequency (10.1%), lower tessitura (33.9%) and twice the rate of head movement rates as compared to AS, thus demonstrating specific responses in each situation. For call duration there was significant interaction between treatment and sex. Additionally, there were differences due to sex, age and coat color. Females and kittens have higher fundamental frequencies may be due to anatomical characteristics. Results also suggest a relationship between coat color and temperament, as solid-colored coated cats presented higher fundamental frequency than other coat colors. Overall, vocal parameters and behavioral signals seem useful indicators for studying the emotions of cats in different situations; further studies are warranted to understand the subtleties of cat vocalization across sex, age and coat color.

Keywords: 1.Acoustic analysis; 2.Acoustic parameters; 3.Animal welfare; 4.Behavioral signs; 5.Emotion assessment; 6. Vocal communication

2.1 INTRODUCTION

Vocalization is the complex motor pattern composed of adduction and abduction of the vocal folds, the result of an interaction between nervous signals and the mammalian vocal system, consisting of the respiratory system and vocal tract (THOMS e JÜRGENS, 1987; FITCH et al., 2002). The active generation of sounds with specific organs is an expression of distinctive inner states in animals, which may occur spontaneously or may be a more direct result of an external event. Therefore, the acoustic response can be identified as a subjective comment, by an individual, about his or her own internal state (GRANDIN, 1998; WATTS e STOOKEY, 2000). Thus, the communication is propagated by acoustic waves that convey a wide range of information about the communicator, including his or her emotional, motivational and physiological state (FRIEL et al., 2016).

Cat vocalization is a natural behavior used for communication, as in other species. Domestic cats developed a vocal repertoire that is more extensive, variable and complex than most other carnivores, which can be explained by their social organization, nocturnal activity, long period of association between mother and kitten, and the dependence of food supply, intentionally or not, by humans (LITTLE, 1993). However, excessive vocalization may be related to some health problems or, alternatively, it may be learned through positive reinforcement or as a means of attracting human attention (BEAVER, 2003; YEON et al, 2011; SAITO e SHINOZUKA, 2013).

Acoustic signalling is a resource for welfare and behavior analyses in pets. Classifying types of vocalization by the situations which evoke them can contribute to the definition of response categories closely linked to the events (BROWN et al., 1978). Thus, different vocal responses can be reliably emitted in different circumstances, reflecting different emotional or motivational states of the animals (BRIEFER, 2012). This can provide useful tools to investigate the motor, perceptual, motivational and social development of the cat (BROWN et al., 1978). Additionally, this may become an extremely relevant tool for the assessment of cat welfare.

Contexts categorized as pleasant are attributed to situations related to feeding and affiliation, while aversive ones can be associated with the condition of cat maintenance in unknown environments, such as automobiles, where aggressive and defensive sounds may be emitted (NICASTRO e OWREN, 2003). However, little is known about the phonetic characteristics of these sounds, as published studies report limited number of cats, types of vocalization or methods (SCHÖTZ, 2015). Therefore, the objective of this study was to compare the vocal and behavioral characteristics exhibited by domestic cats in two different situations, either facing an aversive or a pleasant situation.

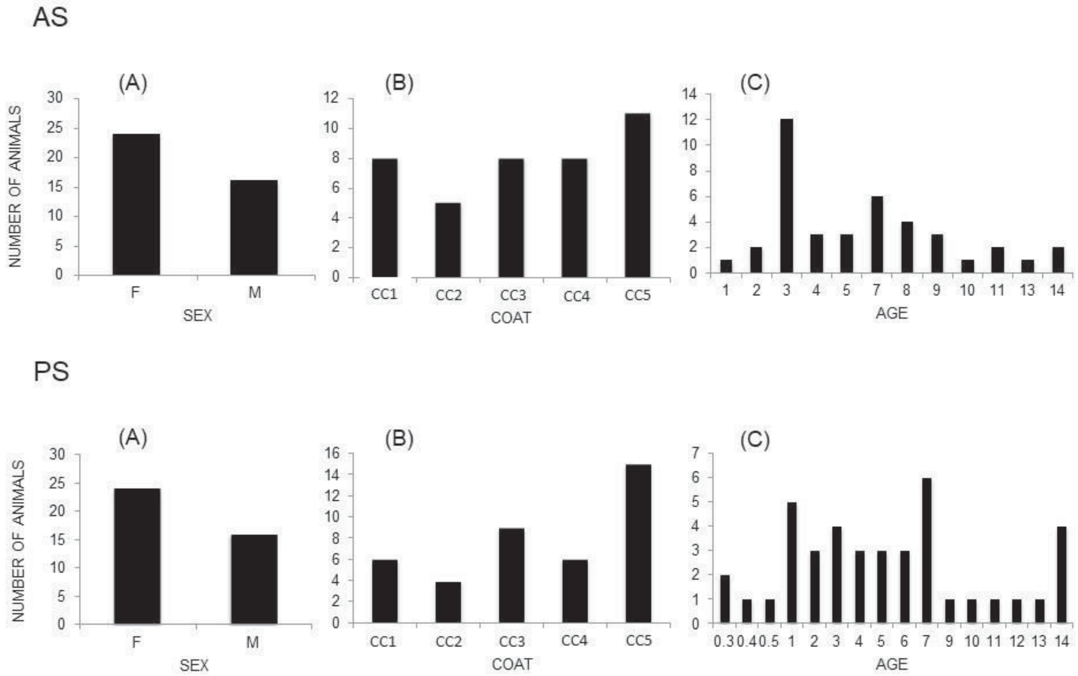
2.2 MATERIALS AND METHODS

2.2.1 Animals, video recordings and management

The cats were initially selected from the availability of their guardians to collaborate with the project. Preliminary tests were carried out with 223 mixed breed home cats living in the city of Curitiba, Paraná, Southern Brazil, from November 30, 2017, to March 22, 2018. The first step was to select 40 animals per group who presented at least five vocal calls during the recording time. As a result, a total of 74 cats (29 males and 45 females) participated in the study. From this total, 68 (26 males and 42 females) were divided into two groups according to the type of situation they were exposed to: aversive (AS) or pleasant situation (PS). The other six animals (3 males and 3 females) participated in both situations, as they fulfilled the required five call criterium for both situations. The AS group was composed of 40 cats aged from four months to 14 years, 24 spayed females and 16 neutered males (FIGURE 1); the 40 cats in the PS group were aged between one and 14 years; 24 were spayed females and 16 neutered males (FIGURE 1). The coat color of the 74 cats was classified into five categories based on TODD (1977) and SFETCU (2014): solid colors (CC1); colorpoint (CC2); striped (CC3); black and white (CC4); calico, tortoiseshell, and tricolor stained white, brown and black (CC5).

FIGURE 1 – DISTRIBUTION OF THE NUMBER OF ANIMALS WITHIN THE AVERSIVE (AS) AND PLEASANT (PS) SITUATIONS ACCORDING TO THE CATEGORIES: (A) SEX, FEMALE (F) AND MALE (M); (B) COAT, SOLID COLORS (CC1); COLORPOINT (CC2); STRIPED (CC3); BLACK AND

WHITE (CC4); CALICO, TORTOISESHELL, AND TRICOLOR STAINED WHITE, BROWN AND BLACK (CC5); (C) AGE.



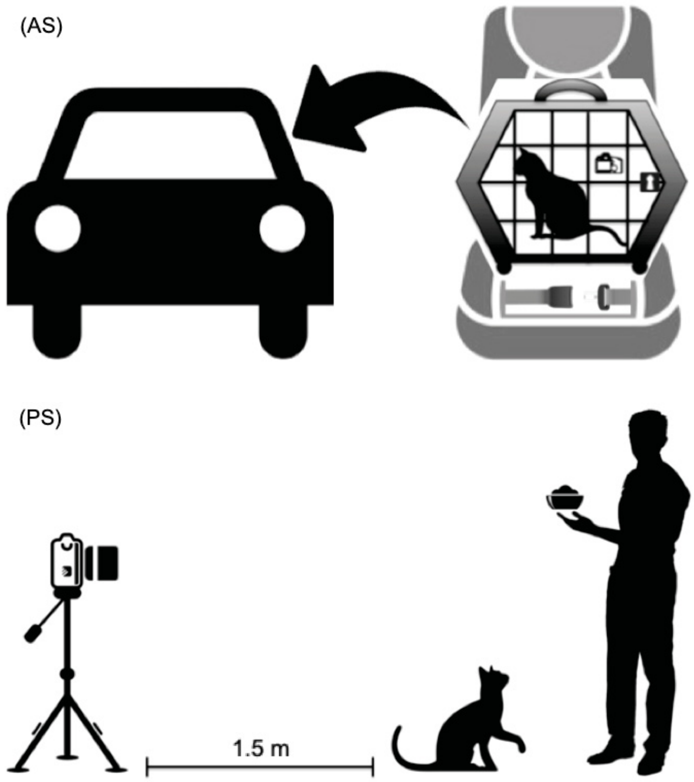
2.2.2 Experimental situations to induce different emotional states

We based our experiment in previous studies by YEON et al. (2011) and FRIEL et al. (2016), to study the difference between cat responses during aversive and pleasant situations. Observations and recordings of behavioral signals and vocal calls were made in two contexts of opposite emotional valence: an aversive situation (AS) and a pleasant situation (PS). In AS, cats were placed in transport boxes at the back seat of a car in the absence of his or her guardian, and conducted to a short drive by the first author. The vocalizations in this case were counted for three minutes, starting from the first vocalization after the car was in movement. On the other hand, in PS, cats were offered their favorite snack by their guardian in their home. The vocalizations in this situation were counted for three minutes, from the first vocal call after recording started. For PS treatment, cats received the snack only after the recording time due to potential changes in the motivation for vocal and behavioral responses.

2.2.3 Vocalization records and behavioral signals

Behavioral signs and individual vocalizations were recorded with a digital camera (Sony Cyber Shot DSC-W610 14.1 Megapixels) for subsequent evaluation. In the AS the camera was coupled to a transport box with dimensions: 55 cm in height, 52 cm in width and 71 cm in length (FIGURE 2). In the PS, the camera was placed on a tripod, directed to the animals, with a distance of approximately 1.5 m from the pair guardian with cat, attached and triggered before the beginning of the test session to allow the animal to adapt to the presence of the camera (FIGURE 2).

FIGURE 2 – SCHEMATIC REPRESENTATION OF TEST SITUATIONS: (AS) AVERSIVE SITUATION WITH THE PLACEMENT AND MAINTENANCE OF THE CAT IN A TRANSPORT BOX, PLACED IN THE BACK SEAT OF A CAR WITH THE CAMERA FIXED TO THE BOX, DURING SHORT TRANSPORT; (PS) PLEASANT SITUATION WITH FAVOURITE SNACK OFFERED BY THE GUARDIAN, WITH THE CAMERA ATTACHED TO TRIPOD, DIRECTED TO THE PAIR CAT WITH GUARDIAN, IN A DISTANCE OF APPROXIMATELY 1.5 M.



2.2.4 Vocal and behavioral measures

To analyze the vocalizations, the audio was separated from the video using the Audacity software (version 2.1.3). The vocalization acoustic signal, which was captured at a sampling rate of 44.1 kHz and quantized at 16 bits, was stored in .wav format files on a MacBook Pro computer with which the acoustic analysis was performed. Acoustic analysis of the vocalization signal was performed with the Praat software, version 5.3.55, developed by Paul Boersma and David Weenink, at the Institute of Phonetic Sciences at the University of Amsterdam.

The vocalization variables were calculated separately for each individual. The acoustic parameters analyzed were vocalization rate, represented by the total number of vocalizations calculated in a temporal window of three minutes divided per three (FIGURE 3); frequency of vocal folds vibration or fundamental frequency (F0); intensity; vocal call duration (FIGURE 4); latency, defined as the time between the beginning of the recording and the beginning of the first vocalization (MARTIN et al., 1993); and tessitura, which is the difference between the lowest and the highest F0 value. For all parameters, with the exception of vocalization rate and latency, we analyzed five vocalization events from the 3 min-video recording, to ensure the analysis of the same number of vocalizations for all animals.

FIGURE 3 – INDIVIDUAL VOCALIZATIONS SELECTED FOR ACOUSTIC ANALYSIS; IN THE WINDOW AT THE TOP OF THE FIGURE: WAVEFORM OF THE ACOUSTIC SIGNAL; BOTTOM: CORRESPONDING SPECTROGRAM OBTAINED BY PRAAT SOTWARE.

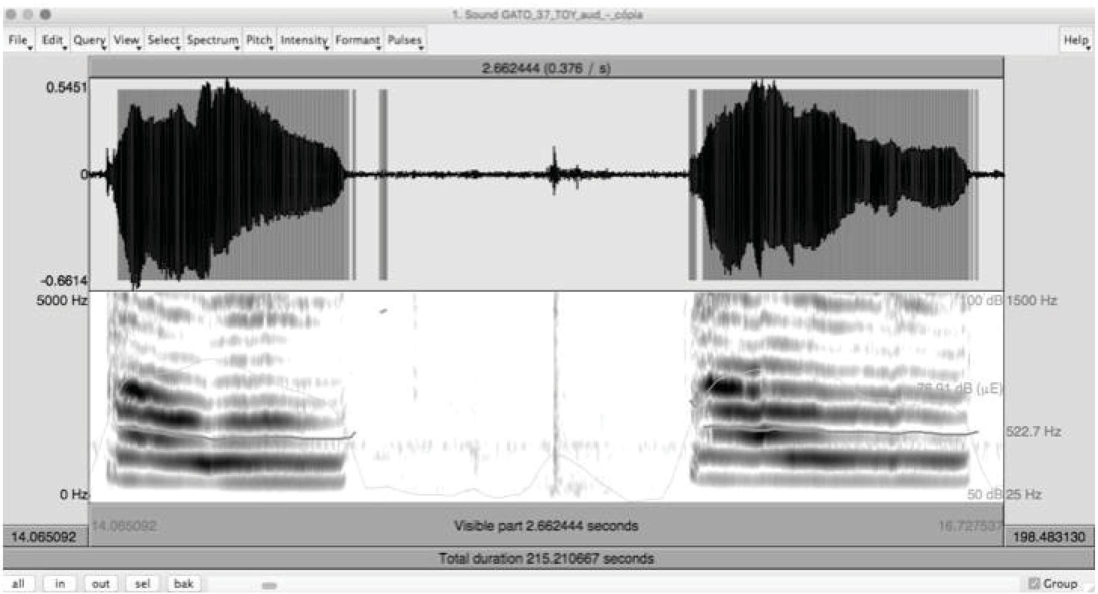
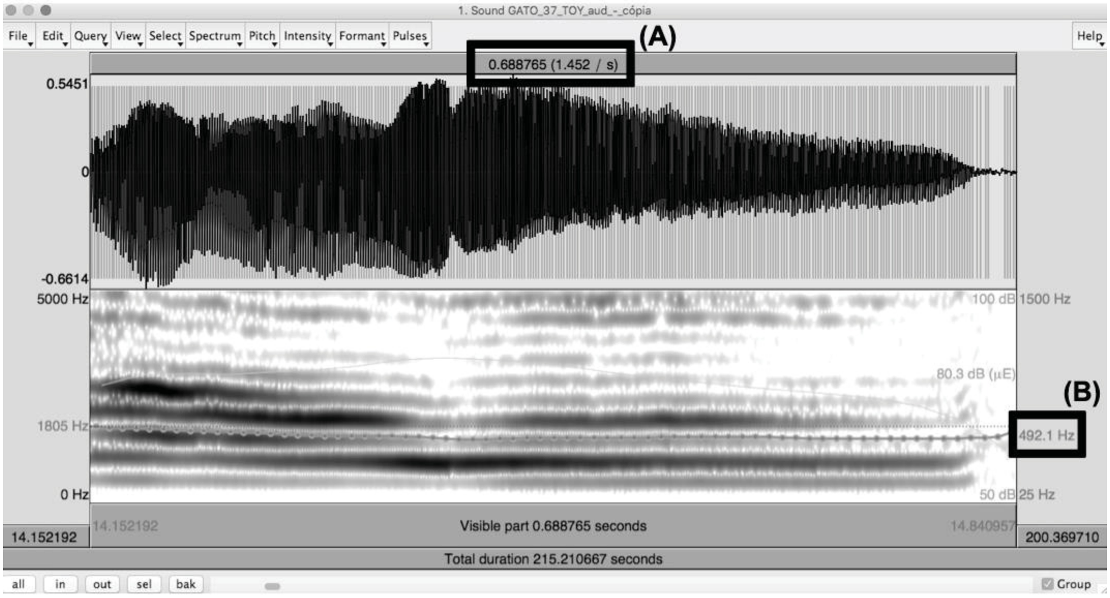


FIGURE 4 - TOP: WAVEFORM OF A CAT VOCALIZATION; SEE TOTAL DURATION OF THE VOCALIZATION INDICATED IN A; BOTTOM: SPECTROGRAM OF A CAT VOCALIZATION WITH FUNDAMENTAL FREQUENCY (F0) TRACED BY DOTTED LINE AND INDICATED IN B.



Behavioral events were analyzed from the records. All behavioral analysis was performed by the first author. In AS group, the behavioral events assessed included tail movements to the right and to the left; body movement to the right and to the left; sitting and recoiling; head movement to the right, to the left and head maneuvering; nose licking; ear movement backwards, to the right, to the left; eye movement; and attempts to escape. In the PS group, the events counted were tail movement to the right, left, up and down; body movement to the right, to the left, sitting and lying down, head movement to the right, to the left, and head maneuvering; nose licking; ear movement to the back, to the right and to the left; and leaning on objects and people, as in this treatment cats were free to interact.

2.2.5 Statistical analysis

The vocalization variables and behavior were submitted to regression analysis, based on generalized linear models. Initially, the five vocalizations per observation period were summarized into a mean value. The explanatory variables were sex, coat (five categories: solid colors - CC1; colorpoint - CC2; striped - CC3; black and white - CC4; calico, tortoiseshell, and tricolor stained white, brown and black - CC5), age and treatment (AS and PS). For vocalization variables, Gamma distribution was considered for the response, due to the asymmetry and the heterogeneous dispersion in the data. Additionally, it was used a logarithmic link function, which provided a better fit. In general, the generalized linear model for the vocalization variables was defined as follows:

$y|x \sim \text{Gamma}(\mu x, \sigma)$, (1)

where μx denotes the mean and σ a dispersion parameter, and:

$\ln(\mu x) = \beta 0 + \beta 1(\text{Sex} = \text{Male}) + \beta 2(\text{Coat} = 2) + \beta 3(\text{Coat} = 3) + \beta 4(\text{Coat} = 4) + \beta 5(\text{Coat} = 5) + \beta 6\text{age} + \beta 7(\text{Situation} = \text{PS})$. (2)

In addition, the interaction effects between treatment and the other factors were tested; when significant, the treatment effect was evaluated separately for each level of the associated factor. We also analyzed the need to model the dispersion parameter in function of the explanatory variables. We had not analyzed the variable intensity of vocalization, since it is affected by the distance between the vocalization origin and recorder, and this distance was consistently different between the aversive and pleasant situations, due to the positioning of the cameras.

Regarding behavior variables, we analyzed the number of movements presented by the animal throughout the experiment. In this case, a negative binomial distribution was used for the response, due to the presence of overdispersion in the data, which made the use of the Poisson distribution unsuitable. The model was specified in a similar way to the regression model applied to vocalization variables, as provided in (1) and (2), replacing the Gamma distribution by the negative binomial, appropriate for the analysis of count data.

The fitted models were validated through graphic analysis of the residuals. The results were presented using the marginal means and respective standard errors. In relation to the variable age, marginal means for the ages of 3 and 8 years were presented, because they configure, respectively, the first and the third sample quartile. Specific notations were used to identify differences between treatments, coat, sex or age. For the coat effect, the descriptive level (p-value) of the tests was corrected due to multiple comparisons.

The analysis was finalized with the study of correlations for the set of vocalization variables and behavior. Correlations were calculated by adjusting the treatment effect and other covariates. As the adjusted variables presented normal distribution, Pearson's linear correlation coefficient was applied. All analyses were performed in statistical software R, version 3.5.1. (TEAM, 2018). The gamlss package was used for model tuning and validation. To obtain the marginal means, the emmeans package was used. Finally, the corrplot package was used in the correlation analysis. For all analyses a significance level of 5% was considered. When comparisons produced results greater than 100%, values are reported in number of times.

2.2.6 Ethical Note

This work is in accordance with the provisions of Brazilian Federal Law No. 11,794, of October 8, 2008, of Decree No. 6.899, of July 15, 2009, and with the norms issued by the National Council for the Control of Animal Experimentation (CONCEA), and was approved by the Ethics Committee on Animal Use (CEUA) of the Agrarian Sciences Sector of the Federal University of Paraná - Brazil, with grade 1 of invasiveness, at the meeting on February 6, 2017, certified with protocol number 055/2017.

2.3 RESULTS

There were differences in vocal parameters as well as in body movements between AS and PS.

2.3.1 Vocal Measures

The treatment effects were significant for F0 and tessitura (TABLES 1-2). The mean F0 was higher in PS than in AS (10.1%) (P=0.011) and, on the other hand, the tessitura was 51.3% higher in AS than in PS (<0.001).

TABLE 1 – ESTIMATED MARGINAL MEANS (EMM), STANDARD ERRORS (SE) AND MULTIPLE COMPARISONS (MC) OF FUNDAMENTAL FREQUENCY (F0) IN HERTZ EXHIBITED BY 74 CATS DURING 80 OBSERVATION PERIODS, ACCORDING TO THE EFFECTS OF SEX, FEMALE (F) AND MALE (M); TREATMENT, AVERSIVE (AS) AND PLEASANT (PS) SITUATIONS; COAT, SOLID COLORS (CC1), COLORPOINT (CC2), STRIPED (CC3), BLACK AND WHITE (CC4), CALICO, TORTOISESHELL, TRICOLOR STAINED WHITE, BROWN AND BLACK (CC5); AND AGE, LESS THAN 3 YEARS, 3 TO 8 YEARS AND MORE THAN 8 YEARS.

Factor	**Category**	**EMM**	**SE**	**p-value**	**MC**
Treatment	PS	568.6	14.0	0.011	NA
	AS	516.2	16.1		

Sex	F	586.8	15.5	<0.001	NA
	M	500.3	15.2		
Age	3 years	590.3	14.0	<0.001	NA
	8 years	506.7	12.0		
Coat	CC1	602.8	27.0	0.039	A
	CC2	560.4	31.8		AB
	CC3	509.7	20.7		B
	CC4	527.4	23.7		AB
	CC5	514.2	18.1		B

TABLE 2 - ESTIMATED MARGINAL MEANS (EMM), STANDARD ERRORS (SE) AND MULTIPLE COMPARISONS (MC) OF TESSITURA IN HERTZ EXHIBITED BY 74 CATS DURING 80 OBSERVATION PERIODS, ACCORDING TO THE EFFECTS OF SEX, FEMALE (F) AND MALE (M); TREATMENT, AVERSIVE (AS) AND PLEASANT (PS) SITUATIONS; COAT, SOLID COLORS (CC1), COLORPOINT (CC2), STRIPED (CC3), BLACK AND WHITE (CC4), CALICO, TORTOISESHELL, TRICOLOR STAINED WHITE, BROWN AND BLACK (CC5); AND AGE, LESS THAN 3 YEARS, 3 TO 8 YEARS AND MORE THAN 8 YEARS.

Factor	Category	EMM	SE	p-value	MC
Treatment	PS	96.5	7.0	<0.001	NA
	AS	146.1	10.2		
Sex	F	139.0	9.7	0.003	NA
	M	101.5	8.0		
Age	3 years	138.8	8.5	<0.001	NA
	8 years	105.1	6.4		
Coat	CC1	135.4	15.7	0.006	A
	CC2	137.9	20.3		A
	CC3	121.7	12.8		A
	CC4	125.5	14.6		A
	CC5	82.8	7.7		B

The effect of sex was significant for fundamental frequency and tessitura (TABLES 2-3). The fundamental frequency for females was 17.2% higher than the fundamental frequency for males, adjusted for the effects of other variables (P<0.001). The tessitura for females was 36.9% higher than the tessitura for males (P=0.003). Also, there was a significant effect of age for fundamental frequency (TABLE 1), tessitura (TABLE 2), and duration (TABLE 3).

TABLE 3 - ESTIMATED MARGINAL MEANS (EMM) AND STANDARD ERRORS (SE) OF DURATION IN SECONDS BY 74 CATS DURING 80 OBSERVATION PERIODS, ACCORDING TO THE EFFECTS OF AGE, LESS THAN 3 YEARS, 3 TO 8 YEARS AND MORE THAN 8 YEARS; AND THE SIGNIFICANT

INTERACTION BETWEEN SEX, FEMALE (F) AND MALE (M), AND TREATMENT, AVERSIVE (AS) AND PLEASANT (PS) SITUATIONS.

Factor	Category		EMM	SE	p-value
Age	3 years		0.81	0.02	<0.001
	8 years		0.71	0.02	
Treatment|Sex	F	PS	0.62	0.03	<0.001
		AS	0.81	0.04	
	M	PS	0.62	0.04	<0.001
		AS	1.02	0.06	
Sex|Treatment	PS	F	0.62	0.03	0.985
		M	0.62	0.04	
	AS	F	0.81	0.04	0.004
		M	1.02	0.06	

The mean fundamental frequency was 16.5% higher for three-year-old cats than for eight-year-old cats (TABLE 1). Tessitura and duration decreased with age. The tessitura was 32.1% higher as well as the duration was 14.1% higher in three-year-old animals when compared to eight-year-old cats (P<0.001) (TABLES 2-3). Regarding the coat color there were significant differences for fundamental frequency and tessitura (TABLES 1-2). Color coat 1 (CC1) cats presented higher fundamental frequency than those with CC3 and CC5, with increases of 18.2 and 17.2%, respectively (P=0.039). The tessitura was lower in CC5 animals than among animals with other coats (P=0.006).

A significant interaction between sex and treatment was observed for the duration of vocalizations (TABLE 3). The mean duration of vocalization was approximately 20.6% lower for females than for males in AS (P=0.004); however, the difference was not significant in the PS group (P=0.985). The difference in the duration of vocalizations for cats in both situations (AS and PS) was significant for both males and females. For males it was on average 64.5% higher for AS than for PS (P<0.001). For females, duration was 30.1% higher in AS than in PS (P<0.001).

The interaction between treatment and coat was significant for vocalization rate (TABLE 4). In CC3, the vocalization rate was 2.2 times higher for AS (P<0.002); in CC4, the vocalization was 4.8 times higher for AS (P<0.001); and in CC5 vocalization rate was 1.8 times higher for AS (P=0.007). The latency variable did not present significant differences for any comparison, with the means for treatments of 7.42 ± 10.8 AS and 6.21 ± 7.37 for PS.

TABLE 4 - ESTIMATED MARGINAL MEANS (EMM) AND STANDARD ERRORS (SE) OF VOCALIZATION RATE PER MINUTE CONSIDERING THE SIGNIFICANT INTERACTION BETWEEN COAT, SOLID COLORS (CC1), COLORPOINT (CC2), STRIPED (CC3), BLACK AND WHITE (CC4), CALICO, TORTOISESHELL, TRICOLOR STAINED WHITE, BROWN AND BLACK (CC5); AND TREATMENT, AVERSIVE (AS) AND PLEASANT (PS) SITUATIONS.

Factor	Category		EMM	SE	p-value
Treatment|Coat	CC1	PS	6.3	1.4	0.127
		AS	9.9	1.9	
	CC2	PS	7.5	2.0	0.189
		AS	12.1	2.9	
	CC3	PS	8.8	1.6	0.002
		AS	19.6	3.7	
	CC4	PS	4.0	0.9	<0.001
		AS	19.3	3.7	
	CC5	PS	8.1	1.1	0.007
		AS	14.6	2.4	
Coat|Treatment	PS	CC1	6.3	1.4	0.739
		CC2	7.5	2.0	
		CC3	8.8	1.6	
		CC4	4.0	0.9	
		CC5	8.1	1.1	
	AS	CC1	9.9	1.9	0.602
		CC2	12.1	2.9	
		CC3	19.6	3.7	
		CC4	19.3	3.7	
		CC5	14.6	2.4	

2.3.2 Other behavioral responses

There was a significant difference between treatments for head movements: the estimated marginal mean for AS was 6.4 ± 0.46 and for PS was 13.1 ± 0.76 head movement per three minute recording; thus, there were events with lower frequency of movements in AS as compared to PS (51.1%) (P<0.001). Ear movements were less prevalent with age, 63.6% higher in cats at three years than in animals at eight years of age (P=0.015) (TABLE 5). There was a significant interaction between sex and treatment for ear movement: females presented 3.2 times higher ear movement rate than males in the AS (P=0.019), while in PS there was not a significant difference between females and males. There was a lower rate of ear movement in the AS than in the PS for males (76.2% lower) (P=0.008), while for females there was not a significant difference between the treatments (TABLE 5).

TABLE 5 - ESTIMATED MARGINAL MEANS (EMM) AND STANDARD ERRORS (SE) OF EAR MOVEMENTS PER THREE MINUTE RECORDING EXHIBITED BY 74 CATS DURING 80 OBSERVATION PERIODS, ACCORDING TO THE EFFECTS OF AGE, LESS THAN 3 YEARS, 3 TO 8 YEARS AND MORE THAN 8 YEARS; AND THE SIGNIFICANT INTERACTION BETWEEN SEX, FEMALE (F) AND MALE (M), AND TREATMENT, AVERSIVE (AS) AND PLEASANT (PS) SITUATIONS.

Factor	**Category**		**EMM**	**SE**	**p-value**
Age	3 years		1.8	0.3	0.015
	8 years		1.1	0.2	
Treatment|Sex	F	PS	1.7	0.5	0.967
		AS	1.6	0.4	
	M	PS	2.1	0.7	0.008
		AS	0.5	0.2	
Sex|Treatment	PS	F	1.7	0.5	0.62
		M	2.1	0.7	
	AS	F	1.6	0.4	0.019
		M	0.5	0.2	

2.4 DISCUSSION

2.4.1 Vocalization and behavioral signals in aversive and pleasant situations

There were differences in the vocal parameters and body movements of cats submitted to the AS and PS, suggesting the existence of an effect of emotions on behavioral responses. In this sense, emotional or motivational states influences on the acoustic characteristics of vocalizations are reportedly reliable and predictable, and can provide an effective way of individual acoustic discrimination within and between types of calls (TAYLOR e REBY, 2010). According to BROWN et al. (1978), in sonographic analysis of cats in six different behavioral situations, there are typical distinctions in the duration of vocal response, intensity and fundamental frequency depending on particular behavioral circumstances.

In our trial, PS cats emitted higher F0 than AS. NICASTRO e OWREN (2003) and FEIGHNY et al. (2006) observed calls with lower frequency in aggressive interactions when compared to those emitted in non-aggressive contexts. Also, MOELK (1944) and MCCOMB et al. (2009) stated that cats can vocalize to his or her guardian to solicit food and to demonstrate food preferences. In this regard, the anticipation context tends to affect the amount of effort and the characteristics of vocalization; thus, the increase in F0 may be related to an intention on drawing attention to the food request. In addition, F0 is important for recipient sensory perceptions, including human interlocutors, and it is the main factor that determines the perceived tone of a voice (KENT e READ, 1992). This relation of appeal to the human sensorial capacities by increasing the vocalization F0 is seen as similar to the response of kittens to their mothers during the period when they fed them (MCCOMB et al., 2009; YEON et al., 2011; SZENCZI et al., 2016). Additionally, if the effort of the cat through the food call is not successful, the emphasis can be transferred to the complaint pattern (MOELK, 1944). Furthermore, calls with higher F0 in PS may have been generated by a motivation for claiming due to the 3 min waiting period to get the snack, counted from the first vocal call. Additionally, if the effort of the cat through the food call is not successful, the emphasis may be transferred to the complaint pattern (MOELK, 1944). Besides presenting higher F0, PS cats presented higher rates of head and ear movements than compared to the AS. SAITO e SHINOZUKA (2013) reported that more than half of the cats responded to human voice by moving their heads and about 30% also responded by moving their ears, with a higher magnitude of response from the movements to the guardian than to strangers. On the other hand, in stressful situations, cats with high levels of cortisol in the plasma showed less locomotion (IKI et al., 2011), similarly to the behavioral responses of AS animals in our results. Therefore, PS vocalization and other behavioral responses may be associated to previous positive interactions between the cats and their guardians.

The AS was associated to calls with higher tessitura, higher vocalization rate and longer duration as compared to the PS, probably due to an intention of requesting attention, because of feelings of powerlessness and need for human help. Stress calls analyzed by BROWN et al. (1978) showed wide variation in frequency distribution, being effective in altering the behavior or calling attention of other cats. According to RAND et al. (2002), vocalization was the second most prevalent behavioral response during stress testing. Also, BRIEFER (2012) reported that emotions of high arousal such as fear are associated with shorter intervals between vocalizations. In addition, higher vocalization rates were associated with high levels of cortisol (IKI et al., 2011), which indicates acute stressful situations. Thus, the vocal call parameters observed in AS group evidenced stress.

2.4.2 Interaction of sex, age and coat color in vocalization and behavioral signals

There were effects of sex, age and coat color of the animals. The females presented vocalizations with higher F0 values, higher tessituras and shorter durations. The resonant frequencies in Hz are directly related to the size of the vocal folds. If the length of the vocal folds is doubled, the frequencies will assume lower values, that is, larger vocal folds have lower tones. In order to understand vocal call variations, it is possible to adapt the methods from human speech studies (TAYLOR e REBY, 2010), since the mechanism of vocal production is similar in humans and other mammals (BRIEFER, 2012). Thus, women have voices 1.7 times higher in F0 than men (KENT e READ, 1992; BARBOZA e DE ARAÚJO CARVALHO, 2010); in general, male individuals have larger vocal folds than female representatives, with a consequent lower F0 production. Additionally, F0 may be correlated with the size of the vocal tract: in baboons and humans, males are larger than females and have lower F0 (PFEFFERLE e FISCHER, 2006; PUTS et al., 2006). Comparatively, in domestic cats, male cats tend to be 20% larger and heavier than females (TAN e KUTLU, 1993; PONTIER et al., 1995). Therefore, the higher F0 values observed in female cats in our study seem compatible with the overall anatomical differences between female and male cats.

Younger animals manifested higher F0, tessitura and duration values when compared to older animals. Based on the Acoustic Theory of Speech Production (FANT, 1970), children have shorter vocal folds in comparison to human adults; therefore, extending this hypothesis to our results, kittens may have shorter vocal folds in comparison to adult cats. Thus, considering the relationship between vocal folds length and F0 frequency, the results we obtained in our analyses are predictable (KENT e READ, 1992; BARBOZA e DE ARAÚJO CARVALHO, 2010; TAYLOR AND REBY, 2010). Also, vocalizations with greater duration may be related to a need for greater appeal, translated into longer vocal communication, which is vital for the initial survival of the young, suggested for species in which the mother hides her offspring and the young must reveal their exact location (SZENCZI et al., 2016). In human animals, children present a slow rate of speech production with broader utterances, (KENT e READ, 1992). Therefore, the vocalization of young cats in a more lasting way may be related to the motor immaturity of the speech, being translated in slowness of the vocalization. Furthermore, regarding high rates of ear movements, younger cats are more motivated to react (CURTIS, 2008) and tend to be more active and alert than older animals.

An unplanned comparison produced an interesting result: the differences in F0 values and vocalization rates among the cats of different coat colors. Assays and observations relating to animal temperament and coat color were performed on some species, such as rats (KEELER e KING, 1942; COTTLE e PRICE, 1987), foxes (BELYAEV, 1979; BELYAEV et al.,1981), dogs (PODBERSCEK & SERPELL, 1996; BENNETT e HAYSSEN, 2010; FRATKIN e BAKER, 2013), cattle (TŐZSÉR et al., 2003), horses (RIEDER, 2009) and cats (DELGADO et al., 2012; STELOW et al., 2016). Overall, it seems to be a correlation between hair characteristics and morphological, physiological and behavioral trends in mammals. Some genes that affect coat color also manifest in all parts of the body, producing morphological changes. For instance, TRUT et al. (2009) reported morphological changes, such as coat color, in domesticated foxes over generations of selection for behavior. Then, coat mutations can be associated with physiological natures and condition expression of unique trends in behavior (KEELER, 1947). The correlation between coat color and temperament may be due to a common biochemical synthesis between melanin and the catecholamines group of neurotransmitters (PODBERSCEK e SERPELL, 1996).

Solid color cats emitted vocalizations with higher F0 values than striped cats and calico, tortoiseshell, and tricolor stained white, brown and black cats. Also calico, tortoiseshell, and tricolor stained white, brown and black cats had higher vocalization rates than black and white cats during PS. In light of understanding this relationship, we compare previous studies regarding coat and temperament of cats. MOURA (2008) stated that solid black cats are adaptable and obedient, solid white coat are non-possessive and the black and white cats are less active. In addition, DELGADO et al. (2012) claimed that three colors coat cats were considered more intolerant than black and white cats, which were characterized as more friendly. According to STELOW et al. (2016), the tortoiseshell colored cats were more aggressive. Thus, high F0 is associated with calmer individuals, such as solid colored when compared to three-colored cats. More research with behavioral monitoring is needed to help understanding the relationship between coat color and behavior of domestic cats, especially considering the cumulating evidences for an association between these characteristics.

2.5 CONCLUSION

Our results strongly suggest that acoustic parameters, such as F0, tessitura, duration and rate of cat vocalization, as well as behavioral signs, like head and ear movements, can be good indicators for better perceiving and understanding cat responses to different situations. Cats exposed to PS have higher F0, lower tessitura, shorter call duration and twice the rate of head movements in comparison to AS. Vocal indicators seem also capable of transmitting information related to sex and age: in our study, females emitted higher F0 and tessitura than males, and young cats had higher F0 and tessitura, but longer duration of calls. Additionally, our study suggests a relationship between coat pattern and vocalization, as solid-colored coated cats presented higher F0 than others. Overall, the comprehension of the specific vocal and behavioral response characteristics expressed by domestic cats in relation to positively or negatively valenced situations seem useful for the assessment of cat welfare, provided detailed information regarding age, sex and coat colour is considered.

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3 VOCALIZATION AS AN INDICATOR OF EMOTIONAL VALENCE: THE CASE OF COW-CALF SEPARATION AND REUNION

RESUMO

A vocalização pode comunicar o estado emocional de alguns animais. Este estudo teve como objetivo comparar a vocalização e os sinais comportamentais de pares de vacas e bezerros em situações emocionalmente negativas e positivas, bem como as interações de grupo genético, categoria de paridade e sexo do bezerro. Quarenta e duas vacas de corte - 16 primíparas e 26 multíparas; filhas de mães mestiças Taurinas, 16 Limousin (LIMO), 15 Charolês (CHAR), seis Brahman (BRAH) e cinco touros Blond d'Aquitaine (BLON) - e seus bezerros de 24 horas de idade (24 machos e 18 fêmeas), sem separação prévia de suas mães, foram estudados no Sul do Brasil. Gravamos vídeos de 3 minutos para analisar as respostas dos animais a três tratamentos: T1 - vaca e bezerro juntos no curral um (C1) contados a partir do fechamento do portão; T2 - bezerro após retirada de C1, isolado no curral 2 (C2) não vendo a vaca, mas a ouvindo; T3 - bezerro e mãe reunidos em C1. Para vacas e bezerros, foram mensurados a contagem de vocalização por minuto, frequência fundamental (f0), duração da chamada vocal e latência para resposta vocal em T1, T2 e T3, além de outros comportamentos observados. Em vacas T2, as respostas vocais tiveram maior duração para LIMO (24,6%) e CHAR (18,2%) em comparação com T1; 15,7% e 33,6% em comparação ao T3. Em T2, vacas LIMO (114,2Hz) e BRAH (124,6Hz) apresentaram maior f(0). Vacas CHAR e BRAH vocalizaram 2,8 e 5,1 vezes mais frequentemente durante T2 do que T3. As vacas apresentaram maior frequência de movimentos da cauda e cheiraram mais o curral em T2 do que em T1 e T3. Maior frequência de movimento da cabeça durante T2 em comparação com T3 foi observada em vacas. Em T2, os bezerros movimentaram mais a cabeça e o corpo, os movimentos das orelhas foram mais frequentes nos bezerros machos e os movimentos de corpo das vacas também foram mais frequentes. Em geral, frequência fundamental mais alta, chamadas vocais mais longas e mudanças comportamentais foram identificadas em T2, como abanar a cauda, cheirar o curral e movimentos da cabeça e do corpo, sugerindo que estes podem ser indicadores úteis de situação emocionalmente negativa percebida por bovinos.

Palavras-chave: 1.Análise acústica; 2.Avaliação de emoções; 3.Bem-estar animal; 4.Comunicação vocal; 5.Parâmetros acústicos; 6.Sinais comportamentais.

ABSTRACT

Vocalization may communicate the emotional state in some animals. This study aimed to compare the vocalization and behavioural signs by cow-calf pairs in emotionally negative and positive situations, as well as the interactions of genetic group, parity category and calf sex. Forty-two beef cows – 16 primiparous and 26 multiparous; daughters of Taurine crossbred mothers, 16 with Limousin (LIMO), 15 Charolais (CHAR), six Brahman (BRAH) and five Blond d'Aquitaine (BLON) bulls – and their 24 h-old calves (24 males and 18 females), with no previous separation from their mothers, were studied in Southern Brazil. We recorded 3 min-videos to analyse animal responses to three treatments: T1 – cow and calf together in corral one (C1) counted from gate closure; T2 – calf after removal from C1, isolated in corral 2 (C2) not seeing the cow, but hearing her; T3 – calf back with mother in C1. For cows and calves, the vocalization count per minute, fundamental frequency (f0), duration of the vocal call and latency to vocal response in T1, T2 and T3 were measured, as well as other behaviours observed. In T2 cows vocal responses lasted longer for LIMO (24.6%) and CHAR (18.2%) in comparison to T1; 15.7% and 33.6% in comparison to T3. In T2, LIMO (114.2Hz) and BRAH (124.6Hz) cows showed higher f(0). CHAR and BRAH cows vocalized 2.8 and 5.1 times more frequently during T2 than T3. Cows showed higher frequency of tail movements and smelling the corral in T2 than T1 and T3. Higher head movement frequency during T2 as compared to T3 was observed in cows. In T2, calves moved their heads and their bodies more frequently, ear movements were more frequent presented in male calves and cow body movements were also more frequent. Overall, high pitch, longer vocal calls and behavioural changes were identified in T2, such as tail flapping, sniffing the corral, and head and body movements, suggesting these may be useful indicators of emotionally negative situation as perceived by cattle.

Keywords: 1.Acoustic analysis; 2.Acoustic parameters; 3.Animal welfare; 4.Behavioral signs; 5.Emotion assessment; 6. Vocal communication

3.1 INTRODUCTION

The concept of animal welfare involves theories of an animal's natural life, biological functioning and affective states (BROWNING e VEIT, 2020; FRASER et al., 1997). Hence, animal welfare assessment includes health parameters as well as the reactions and behaviours in response to handling and other environmental stimuli, which can be used to estimate the effects of raising methods and procedures on the affective states of the animals (KIM et al., 2019). In this sense, the speculation of emotions in animals is based on various types of experimental and observational evidence resulting from behavioural studies and comparison between physiological and neurobiological processes in humans and nonhumans (WEARY et al., 2017). A tool that can be used to analyse circumstances and degrees of animal welfare and emotions is the vocal response (BOISSY et al., 2007), with programs that perform the measurement of acoustic events, based on variables such as fundamental frequency or pitch and intensity (GRANDIN, 2001; MEEN et al., 2015; VOLODIN et al., 2017; WATTS e STOOKEY, 1999; WATTS et al., 2001; WEARY e CHUA, 2000), as well as the count of vocalization analysed from the recordings. Vocal calls can provide conspecific with meaningful information about the physiology, affective state and physical attributes of the caller (BLANK, 2020). There are many studies with different species showing vocal calls repertoire facing emotionally negative and positive situations, such as cats (FERMO et al., 2019); chickens (Hale et al., 2014); goats (BRIEFER et al., 2014); and piglets (SPINKA et al., 2018). In mammals, vocal communication occurs in mother-infant interactions as one of the initial evolutionary mechanisms that stimulate the development, together with nursing and playing (BRUDZYNSKI, 2014). Regarding cattle, the vulnerability of newborn calf and its need for protection tends to increase maternal responses (TURNER et al., 2020). In beef cattle, maternal behaviour is essential for proper development of newborn calf and to preserve it from threats (Hoppe et al., 2008). Thus, cows recognize their progeny early (KILEY, 1972) and exhibit high levels of care and selectivity, fostering exclusively their own offspring (LE NEINDRE, 1989; MARCHANT-FORDE et al., 2002; NOWAK et al., 2000). The immediate vocal response of the cow facilitates the surveillance and prevents future episodes of separation.

In cattle, vocalization is a common response to one or more endogenous or external stimuli. These stimuli may also be capable of altering muscle tension and action patterns of the vocal apparatus, impacting the parameters of vocal calls in a coherent manner regarding the emotional state of the animal. Specific parameters may suggest the intensity of the experience and the valence of emotions it evokes, which may be negative or positive (BRIEFER, 2012; GRANDIN, 1998). Thus, it seems possible to use these vocal parameters to better understand emotions in cattle and to support initiatives to correct practices that do not promote a harmonious, healthy and comfortable life for animals.

The vocal response measurement during unpleasant handling procedures or pleasant situations may show specific vocal parameters that can be used as indicators of animal welfare (NDOU et al., 2011). In addition, vocalization use as welfare indicator has the advantage of being a totally non-invasive strategy, unlike some physiological measurements (GRANDIN, 1998; GREEN et al., 2019; WATTS e STOOKEY, 2000).

Cattle are gregarious animals who live in interactive groups (CANALI et al., 2001; RUSHEN et al., 1999). Therefore, livestock practices that promote social separation increase behavioural and physiological measures related to the activation of the hypothalamic-pituitary-adrenocortical axis, which suggests that social deprivation has intense psychological effects in cattle (BOISSY e LE NEINDRE, 1997; RUSHEN et al., 1999), with a negative valence (BRIEFER et al., 2015). Similarities between reactions to isolation and painful procedures support that segregation causes significant reduction in the welfare of social animals (WATTS et al., 2001). Cow-calf separation is a stressful stimulus for both animals involved, causing an increase in vocalization, locomotor activity, cortisol concentration, loss of weight and heart rate (FLOWER e WEARY, 2001; STĚHULOVÁ et al., 2008; ORIHUELA e GALINA, 2019). In dairy cows and calves, artificial maternal separation circumstances also seem related to negative states, such as anguish, fear and frustration (DAROS et al., 2014; HOPSTER e BLOKHUIS, 1994) and cause an increase in the vocalization count (PROBST et al., 2014). On the other hand, emotionally positive situations present less behavioural responses, like the cow-calf reunion, a situation characterized by a likely reduction in suffering (BOISSY e LE NEINDRE, 1997; LEFCOURT e ELSASSER, 1995).

Emotions may especially be studied according the intensity and valence when the animal is presented to certain situations that can be measured and associated with neurophysiological, behavioural and cognitive changes (ANDERSON & ADOLPHS, 2014; BACIADONNA et al., 2018; BRIEFER, 2012; PAUL e MENDL, 2018). In this regard, as cattle express different vocalizations depending on the context or emotional state (WATTS e STOOKEY, 2000), vocal call variables and body reactions may characterize both hostile and emotionally positive circumstances, as well as being used as potential indicators of emotional valence, excitement grades and, finally, animal welfare status. Indicators of stressful and frustrating situations are reliable components associated with negative valence and high arousal, while contentment indicators may reliably be associated with positive valence and low arousal (MENDL et al., 2010). Therefore, the objective of this study was to compare the vocal parameters and selected behavioural signs expressed by cattle in three different situations, including emotionally negative and positive situations, as well as the interactions regarding genetic group, parity category and calf sex.

3.2 MATERIALS AND METHODS

3.2.1 Ethical note

This study was performed in accordance to Law 11,794, of October 8, 2008, Decree 6,899, of July 15, 2009, and the directions of the National Council for the Control of Animal Experimentation (CONCEA). This study was approved by the Ethics Committee on the Use of Animals (CEUA) of the Agricultural Sciences Sector of the Federal University of Paraná, Brazil, with degree one of invasiveness, at a meeting on 06/02/2017, certified by protocol number 048/2017.

3.2.2 Animals

The study was carried out using beef cattle dams and calves raised for the purpose of reproduction and fattening. A total of 84 animals (42 pairs) participated in the study. Dams were all daughters of taurine crossbred mothers. As for the male parental genetics, four different bull breeds were included: 16 Limousin (LIMO), 15 Charolais (CHAR), six Brahman (BRAH) and five Blond d'Aquitaine (BLON). There were 16 primiparous and 26 multiparous cows. The 42 calves (24 male and 18 female) were 24 hours old and were classified according to dam genetics; the calves had no experience with the corrals or with separation from their dams. The experiment was conducted in Canoinhas, Santa Catarina, South Brazil, at Fazenda Nova Esperança (20°09'40, 25” S; 50°32'39,78” O), from April 17 to July 17, 2018.

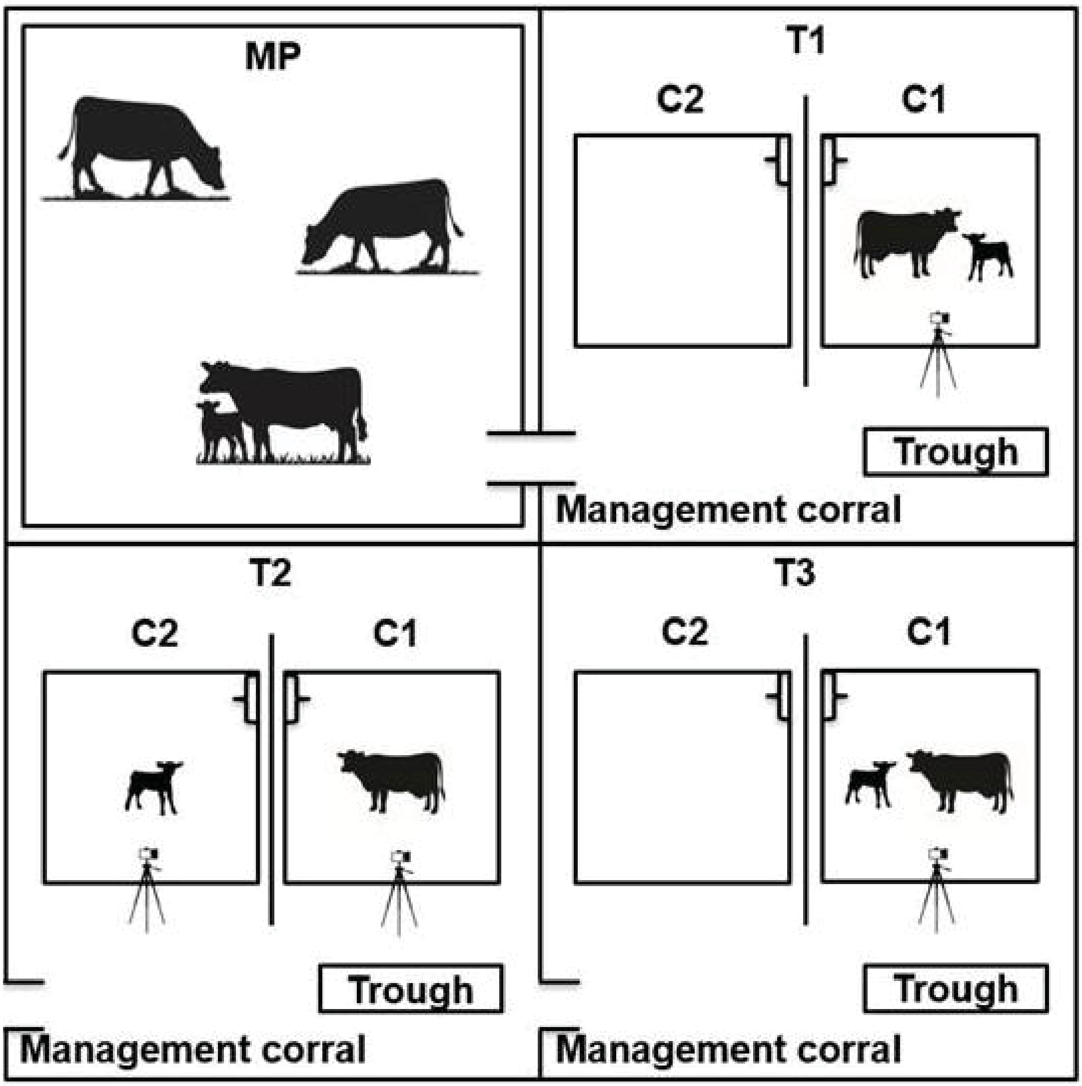
3.2.3 Management of emotional expressions

We used the concepts of WEARY e CHUA (2000) to understand dam and calf reactions facing separation, and SANDEM e BRAASTAD (2005) for the responses provoked by emotionally negative and positive situations, such as their separation and reunion. In this experiment, we considered animal emotional valences as evidenced by physiological and neurobiological responses to a stimulus as compared with human emotional contexts (WEARY et al., 2017). The cow-calf pairs were video recorded during the puerperium period and simultaneously with the procedures of calf navel disinfection and identification. The management for cleaning and disinfection of the navel was performed according to ROBINSON et al. (2015), and calf identification followed information from DA COSTA e CROMBERG (1998). Both procedures were performed after filming to avoid interferences with the reactions to separation.

The pregnant cows in the final third of gestation were housed in a maternity paddock (MP) (FIGURE 5) of 10 hectares, with access to fresh water, fertilized native pasture, and ad libitum concentrated and mineral supplementation inside the handling corral. In the first 24 hours postpartum, the cow accompanied by the calf when entering the stable to feed, were managed at the corral 1 (C1), where they stayed for three minutes counted from corral gate closure for the first recording of behavioural reactions (T1) (FIGURE 5). In treatment 2 (T2), the calf was removed from C1 by a side gate and isolated for three minutes in corral 2 (C2), which was immediately next to the C1, without visual contact with its mother due to a board blocking the view between the two areas, but being able to hear her and be heard. This phase of study represented the emotionally negative situation for both cow and calf and, during this treatment simultaneous video captures were taken of both animals. Subsequently, the calf returned to C1 with his or her mother, staying there with her for the last 3-min recording (T3), which was defined as the emotionally positive situation.

FIGURE 5 - REPRESENTATION OF THE RECORDING SITUATIONS USED TO INDUCE DIFFERENT EMOTIONAL STATES; MATERNITY PADDOCK (MP) IS ADJACENT TO THE MANAGEMENT CORRAL WITH PREGNANT AND POSTPARTUM COWS; TREATMENT 1 (T1): THE DAM AND CALF TOGETHER AT CORRAL ONE (C1); TREATMENT 2 (T2): SIMULTANEOUS COW-CALF RECORDINGS IN DIFFERENT CORRALS DURING THE SEPARATION PERIOD (C1

AND C2); TREATMENT 3 (T3): THE REUNION OF BOTH AT C1; EACH RECORDING LASTED THREE MINUTES.



3.2.4 Vocal responses and body posture reactions

The vocalizations and other behavioural signals were recorded by two digital cameras (Sony Cyber Shot DSC-W610 14.1 Megapixels) for later analysis of the audios and footage at the laboratory. There were hidden cameras on the side of corrals C1 and C2 (FIGURE 5). The cameras were placed on tripods and were turned on before the animals arrived at each corral, in a position allowing for the capture of all movements within the area.

Audacity software (version 2 .1.3) was used to extract the audio from the video. The acoustic signal was sampled at a rate of 44.1 kHz, quantized to 16 bits and stored in .wav format files on a MacBook Pro computer. Then, it was analysed using the software Praat (version 5.3.55), which is a free software developed by Paul Boersma and David Weenink (2013), at the Center for Phonetic Sciences at the University of Amsterdam. We based our analysis on studies of GREEN et al., (2019), KIM et al. (2019), VOLODIN et al. (2017) and YAJUVENDRA et al. (2013).

The vocalization variables and other behavioural signs were evaluated separately for each calf and each cow. The acoustic parameters included were the vocalization count (BOISSY e LE NEINDRE, 1997; FLOWER e WEARY, 2001; MARCHANT-FORDE et al., 2002; SOLANO et al., 2007; WATTS et al., 2001), represented by total number of vocalizations calculated in a temporal window of three minutes divided per three; the frequency of vocal fold vibration or fundamental frequency (f0) (PADILLA DE LA TORRE et al., 2016; RUSHEN et al., 1999; WATTS e STOOKEY, 1999); the duration of the vocal call (KILEY, 1972; DOYLE e MORAN, 2015). Also, latency, which was in this study defined as the time lapse between the beginning of the video-recording and the first event of vocalization (MARTIN et al., 1993), was analysed separately for each treatment or situation: in T1, the situation evaluated was the entrance into C1; for T2, for the cow was calves' exit and for calves, it was the entrance into C2 (social isolation from their mothers); in T3, for both cow and calves was the reunion in C1.

Studies associate vocalization and behaviour signals depending on the situation (JOHNSEN et al., 2018; GREEN et al., 2020). The behavioural assessment consisted of quantifying specific movements for later association with vocal repertoire according to the situations provided. The behaviour signals observed were assigned based on previous studies regarding behaviour collection of cows and calves (TABLE 6). In T1 and T3, attempts to escape (CRONEY et al., 2000); defecating; urinating (MUNKSGAARD et al., 1997); suckling (NAKANISHI et al., 1993; SOLANO et al., 2007; SOMERVILLE e LOWMAN, 1979); calf and cow approaching (ENRÍQUEZ et al., 2010); head butting (GIBBONS et al., 2009; STRICKLIN et al., 1980), sniffing calf or cow (JENSEN, 2011; LIDFORS, 1996); licking calf or cow (DE PASSILLÉ et al., 1995a; STEELE, 2019; TURNER et al., 2020); licking the side of the corral (STĚHULOVÁ et al., 2008); body movement, from step counting (HALEY et al., 2005); tail flapping, measured as each movement of the tail away from the base until return to the relaxed position (MIEDEMA et al., 2011); ear movement; and head movement given by slowing up, down or to one side away from the original position (MARCHANT-FORDE et al., 2002). For T2, the indicators were attempt to escape, defecating, urinating, tail flap, head butting, licking the side of the corral, body movement, ear movement and head movement. All vocalizations were analysed by the first author and the other behavioural signs were analysed from the video recordings by the first author and two interns from the Animal Welfare Laboratory, Federal University of Paraná. The two interns received previous training on the recognition and counting of each movement. Then, the first author checked a random sample and her counting results were equal to those by interns, which was expected due to the objectivity of the countings.

TABLE 6 - VOCAL VARIABLES AND BEHAVIOURAL SIGNS MEASURED IN EACH TREATMENT.

Treatments	Cow	Calf
T1 and T3	- Vocalization count, fundamental frequency, duration and latency to call. - Attempts to escape, defecating, urinating, suckling, cow approaching calf, head butting, sniffing calf, licking calf, licking the side of the corral, body movement, tail flapping, ear movement and head movement.	- Vocalization count, fundamental frequency, duration and latency to call. - Attempts to escape, defecating, urinating, suckling, calf approaching cow, head butting, sniffing cow, licking cow, licking the side of the corral, body movement, tail flapping, ear movement and head movement.
T2	- Vocalization count, fundamental frequency, duration and latency to call. - Attempt to escape, defecating, urinating, tail flap, head butting, licking the side of the corral, body movement, ear movement and head movement.	- Vocalization count, fundamental frequency, duration and latency to call. - Attempt to escape, defecating, urinating, tail flap, head butting, licking the side of the corral, body movement, ear movement and head movement.

3.2.5 Statistical analysis

Data analysis was based on marginal models and the model parameters were estimated through generalized estimation equations (GEE). The behaviour variables were considered as the responses, whereas the experimental treatment was the predictor of main interest. We have used a *quasi-Poisson model* to take into account the data overdispersion. Furthermore, an exchangeable correlation structure was considered to lead with the non-independent repeated measures within animals, and a robust sandwich estimator for the standard errors was applied to prevent possible model misspecifications.

Besides the experimental treatments, the following covariates were also included in the regression models: genetic group, parity category and calf sex. All interaction effects involving the experimental treatments were investigated, aiming to assess different isolation effects among subgroups defined by the covariates. All statistical analysis was performed in *R statistical software*, version 3.6.1, through the *geepack* and *emmeans* libraries. The results are presented as estimated rates, their respective standard errors and confidence intervals (95%). The estimated rates were obtained by averaging the effects of the remaining covariates. The *Tukey* method, which controls the significance level when carrying out multiple tests, was used to ensure a global significance level of 5% in our tests.

The variables describing vocalizations, i.e. pitch, and duration were analysed through linear mixed models, considering the effects of treatments and the remaining covariates, besides a normal random effect of animal. Once again, possible interaction effects were investigated, aiming to evaluate different effects of treatment for distinct levels of the covariates. In the cases where the model residuals did not present normality, a logarithmic transformation was applied. For the other response variables (frequency and latency), marginal models fitted by generalized estimation equations were used, as mentioned before on the behavioural analysis. Results are presented as marginal estimated means or rates, along with their corresponding confidence intervals (95%). All conclusions are based on a significance level of 5%. The logarithmic transformation was only necessary in the analysis of the pitch when we joined vocalizations with open and closed mouths.

3.3 RESULTS

In T1, dams took 4.5 times longer to emit the first vocalization as compared to T3 (3.9%) ($P < 0.05$) (TABLE 7).

TABLE 7 - ESTIMATED MEANS AND STANDARD ERRORS OF ACOUSTIC AND BEHAVIOURAL RESPONSES CONSIDERING THE MAIN EFFECTS OF TREATMENTS, ARRIVAL AT THE CORRAL (T1), COW-CALF SEPARATION (T2) AND THEIR REUNION (T3), PRESENTING INDICATORS FOR WHICH THE INTERACTIONS WERE NOT SIGNIFICANT.

Variables	Treatments*		
	T1	T2	T3
Latency, s	22.43^{a} (4.08)	12.14^{ab} (3.38)	5.02^{b} (1.59)
Tail flapping (cow), frequency	10.5^{a} (1.2)	26.9^{b} (3.6)	9.1^{a} (1.2)
Cow approaching calf, frequency	1.5^{a} (0.2)	NA	2.5^{b} (0.4)
Cow sniffing corral, frequency	3.8^{a}	8.5^{b}	1.9^{c}

	(0.5)	(1.0)	(0.3)
Calf head movement, frequency	11.7[a]	21.8[b]	10.2[a]
	(1.1)	(1.6)	(0.9)
Calf body movement, frequency	48.3[a]	100.7[b]	50.9[a]
	(4.9)	(9.0)	(5.6)

* Different letters indicate significant differences between treatments (P <0.05); NA = not applicable.

A significant interaction effect between treatment and genetic group was observed for duration, pitch and vocalization count. CHAR and LIMO cows had longer vocal calls in the isolation scenario than in T1, 18.2% and 24.6% longer, respectively. Also, vocal calls emitted by cows were 33.6% and 15.7% longer in T2 than T3 (P <0.05) (Table 3). In addition, in T2 cows emitted calls with 13.2% higher pitch than in T1, 5.4% than in T3 for LIMO, and 28.1% higher than T1 for BRAH genetics. The pitch in T1 was 6.9% lower than T3 for LIMO, and 17.1% lower for BRAH (P <0.05) cows. In addition, the number of vocalizations was higher in T2 than T1 for CHAR (5.2 times), LIMO (2.5 times) and BRAH (8 times) cows. Furthermore, BLON and CHAR cows vocalized 40.8% and 46.4% less in T1 when compared to T3. The comparison between T2 and T3 showed a higher number of vocal calls for CHAR (2.8 times) and BRAH (5.1) cows (P <0.05) (TABLE 8).

TABLE 8 - ESTIMATED MEANS AND STANDARD ERRORS OF VOCALIZATION AND OTHER BEHAVIOURAL VARIABLES CONSIDERING THE SIGNIFICANT INTERACTION BETWEEN T1, T2 E T3 TREATMENTS AND GENETIC GROUPS: BLONDE D'AQUITAINE (BLON), CHAROLAIS (CHAR), LIMOUSIN (LIMO) AND BRAHMAN (BRAH), AND BETWEEN TREATMENTS (T1, T2, T3) AND CALF SEX: FEMALE (F) AND MALE (M), PER TOTAL OBSERVATION TIME OF THREE MINUTES.

Variables	**Treatments**	**Genetic group***				**Sex***	
		BLON	**CHAR**	**LIMO**	**BRAH**	**F**	**M**
Duration, s	T1	1.16^{a},(0.13)	1.21^{a},(0.09)	1.18^{a},(0.08)	1.23^{a},(0.15)	NS	NS
	T2	1.27^{a},(0.13)	1.43^{b},(0.07)	1.47^{b},(0.06)	1.23^{a},(0.10)	NS	NS
	T3	1.30^{a},(0.12)	1.07^{a},(0.08)	1.27^{a},(0.07)	1.08^{a},(0.13)	NS	NS
Fundamental frequency, Hz	T1	108.1^{a},(10.5)	111.9^{a},(6.2)	100.9^{a},(5.0)	97.3^{a},(8.6)	NS	NS
	T2	112.3^{a},(10.8)	118.6^{a},(5.9)	114.2^{b},(5.3)	124.6^{b},(9.3)	NS	NS
	T3	112.2^{a},(10.7)	113.4^{a},(5.9)	108.4^{c},(5.0)	117.3^{b},(9.7)	NS	NS
Vocalization count, frequency**	T1	2.9^{a},(1.3)	1.5^{a},(0.5)	2.7^{a},(0.8)	1.2^{a},(0.9)	NS	NS
	T2	3.3^{ab},(1.4)	7.8^{b},(1.3)	6.8^{b},(1.8)	9.6^{b},(2.6)	NS	NS
	T3	4.9^{b},(2.2)	2.8^{c},(0.6)	4.3^{ab},(0.7)	1.9^{a},(0.7)	NS	NS
Tail flapping (calf), frequency	T1	21.4^{a},(13.9)	25.4^{a},(9.9)	4.0^{a},(2.2)	5.3^{a},(2.9)	NS	NS
	T2	41.4^{a},(20.7)	62.8^{a},(12.4)	29.6^{b},(9.6)	91.3^{b},(28.8)	NS	NS
	T3	6.6^{b},(2.7)	24.7^{a},(12.2)	6.3^{a},(3.2)	15.0^{c},(7.8)	NS	NS
Sniffing calf, frequency	T1	0.9^{a},(0.2)	1.8^{a},(0.6)	1.1^{a},(0.3)	4.2^{a},(1.2)	NS	NS
	T2	NA	NA	NA	NA	NA	NA
	T3	3.4^{b},(0.5)	3.3^{a},(0.5)	3.4^{b},(0.4)	4.7^{a},(0.8)	NS	NS
Sniffing the corral (calf), frequency	T1	1.2^{a},(0.5)	3.0^{a},(0.7)	1.5^{a},(0.5)	2.7^{a},(1.0)	NS	NS
	T2	7.2^{b},(1.5)	7.0^{b},(1.1)	9.8^{b},(1.6)	12^{b},(3.7)	NS	NS
	T3	0.4^{a},(0.2)	1.3^{c},(0.4)	1.6^{a},(0.5)	2.2^{a},(1.0)	NS	NS
Head movement (cow), frequency	T1	15.4^{a},(2.0)	18.4^{a},(1.8)	19.5^{ab},(1.9)	29.3^{a},(4.8)	NS	NS
	T2	20.3^{a},(5.9)	27.4^{b},(1.9)	23.6^{a},(1.9)	30.2^{a},(7.2)	NS	NS
	T3	15.6^{a},(2.3)	14.5^{a},(1.7)	16.7^{b},(1.8)	17.4^{b},(5.2)	NS	NS
Body movement (cow), frequency	T1	17.7^{a},(3.9)	27.6^{a},(3.7)	23.9^{a},(5.5)	33.9^{a},(5.6)	NS	NS
	T2	37.2^{b},(8.3)	55.8^{b},(6.6)	43.1^{b},(6.5)	60.0^{b},(22.8)	NS	NS
	T3	25.1^{b},(6.5)	18.1^{a},(3.7)	14.1^{a},(2.3)	26.5^{a},(5.2)	NS	NS
Ear movement(calf), frequency	T1	NS	NS	NS	NS	23.1^{a},(3.8)	20.7^{a},(3.0)
	T2	NS	NS	NS	NS	26.1^{a},(3.2)	34.7^{b},(2.7)
	T3	NS	NS	NS	NS	20.1^{a},(3.0)	18.4^{a},(1.9)
Ear movement (cow), frequency	T1	NS	NS	NS	NS	51.4^{ab},(8.3)	49.8^{a},(4.9)
	T2	NS	NS	NS	NS	52.2^{a},(4.6)	61.8^{b},(4.4)
	T3	NS	NS	NS	NS	43.2^{b},(4.2)	60.6^{b},(5.0)

* Different letters indicate significant differences between treatments for racial ancestry and for sexes (P <0.05); NA = not applicable; NS = not significant;
**Number of vocalizations per minute.

3.3.1 Behaviour assessment

A significant difference was noted in the main treatment effects for tail flapping in cows, there was a higher frequency of movement in T2 than T1 (2.6 times) and T3 (3.0 times) (P <0.05). Dams approached calves more often during T3 when compared to T1 (66.7%) and sniffed the corral more often in T2 than in T1 (2.2 times) and T3 (4.5 times). Also, in the moments that cows and calves were together, dams sniffed the corral less frequently in T3 than T1 (50%) (P <0.05) (Table 2). Furthermore, the results showed that during T2, calves exhibited more head movements when compared to T1 (86.3%) or T3 (2.1 times). In addition, their body movement was more often in T2 than in T1 (2.1 times) and in T3 (97.8%) (P <0.05) (TABLE 7).

The interaction between treatment and genetic group was significant for tail flapping in calves, cows sniffing their offspring, calves sniffing the corral, head and body movements in cows. Calves born from BLON dams flapped their tails more in T2 than T3 (6.3 times) and in T1 than T3 (3.2 times). Calves from LIMO dams showed 7.4 times more movements in T2 when compared to T1 and 4.7 times in relation to T3. Likewise, calves from BRAH dams flapped their tails more often in T2 than in T1 (17.2) and T3 (6.1) and in T1 the flapping rate was 64.6% lower than T3. BLON dams sniffed their offspring 3.8 times more in T3 than T1, and LIMO cows, 3.1 times (P <0.05) (TABLE 8).

Overall, calves from all genetic groups sniffed the corral more frequently in T2 than in T1 (BLON, 6 times; CHAR, 2.3 times; LIMO, 6.5 times; BRAH, 4.4 times) and in T3 (BLON, 18 times; CHAR, 5.4 times; LIMO, 6.1 times; BRAH, 5.5 times). However, Charolais cross offsprings sniffed the corral less in T3 when compared to T1 (56.7%) (P <0.05) (TABLE 8). CHAR, LIMO and BRAH cows moved their heads more frequently in the absence of their offspring than in T3 in 89.0%, 41.3% and 73.6%, respectively. The CHAR cows moved their heads more often in T2 than T1 (48.9%) and BRAH cows 68.4% more often in T1 than T3 (P <0.05) (TABLE 8). Also, cow body movements were more frequent when they were separated from their offspring (T2) than when they were together in T1, with 2.1 times for BLON, 2.0 times for CHAR, 80.3% for LIMO, and 77.0% from BRAH dams. Similarly, in relation to the time cows and calves were together (T2 and T3), cows moved their bodies more in T2 in three genetic groups: CHAR (3.1 times), LIMO (3.1 times) and BRAH (2.3 times). BLON dams moved 41.8% more in T3 than T1 (P <0.05).

There was a significant effect of interaction between treatments and calf sex for ear movements in calves and cows. Male calves showed more ear movements in T2, with 67.6% than in T1 and 88.6% than in T3. Mothers of female calves moved their ears more in T2 than in T3 (20.8%) and mothers of male calves moved their ears less in T1 than T2 (19.4%), and in T3 (17, 8%) (P <0.05) (TABLE 8).

Lastly, the difference in vocalization of calves between treatments was not statistically compared, since 98.2% of their vocal calls occurred in T2, 1.2% of the records occurred in T3 during the reunion and 0.6% in T1. Likewise, statistical comparisons were not possible in the following situations: calf attempts to escape, which occurred 180 times only in T2; and the calf head butting displayed 256 times in T2, once in T1 and eight times in T3. For these variables, the obvious treatment effects were discussed based on descriptive statistics.

3.4 DISCUSSION

The vocal structure and phonatory performance of cattle have already been reported as dependent on the scenario to which animals are exposed (GREEN et al., 2020). In this study, cows took less time to vocalize in T3 than in T1. KILEY (1972) reported that if the calf enters his or her mother's visual field after leaving for some time, the dam immediately vocalizes, which is related to the recognition through vocal calls (BARFIELD et al., 2010; PADILLA DE LA TORRE e MCELLIGOTT, 2017; WATTS E STOOKEY, 2000). Also, individual recognition in gregarious species is fundamental to avoid misdirected parenthood (PADILLA DE LA TORRE et al., 2016) and it also helps in coordinating social behaviours (PADILLA DE LA TORRE et al., 2015). In our results, this urgency of identification between the pair may be related to lower vocal latency values in T3. Also, physical contact between the offspring and dam in T3 may be due to cattle potential acts of communication (WATTS E STOOKEY, 2000) and animal expressions directed at conspecifics, signalling attraction and alertness (MANTEUFFEL et al., 2004; VON KEYSERLINGK e WEARY, 2007), which are recognized as maternal care (TURNER et al., 2020).

The cow-calf separation in T2 resulted in longer vocal calls. Vocalizations can alarm distant receivers and may be evaluated using characteristics such as count and duration (MANTEUFFEL et al., 2004). KILEY (1972) stated that calls made by cattle in social isolation are longer, such as from mother to child or vice versa. Likewise, stressful situations (e.g. separation of couples) are associated with negative valence and high intensity (MENDL et al., 2010). Also, physiological arousal causes an increase in action and tension of the respiratory muscles, inducing longer call durations (BRIEFER, 2012; DOYLE E MORAN, 2015). In addition, GREEN et al., (2020) stated that the longer duration of calls improves vocal transmission. Thus, calls seem to be maintained for a longer time in order to promote greater appeal, reach and draw the attention of the recipient, as well as announce urgent contexts. Our results further indicate the value of longer calls as signal of emotionally negative situations.

According to GRANDIN (1997), there are differences in temperament of cattle according to genetic groups which can affect an animal response to stress. Also, BOISSY E LE NEINDRE (1997) stated that social behaviour can be influenced by breed. In this context, the results show that the emotionally negative situations (T2) generated longer vocal responses in crossbred CHAR and LIMO dams, which is in agreement with BOISSY e LE NEINDRE (1997) statement that social behaviour can be influenced by breed. Also, our findings show that T2 induced LIMO and BRAH cows to emit vocal responses with higher pitch values as compared to other genetic groups.

The results presented different pitches in cattle vocal call as related to emotional valence. The emotional state of the call emitter causes changes in the action of his or her vocal apparatus, which in turn impacts the vocal parameters of the calls (BRIEFER, 2012). Thus, cows produce distinct, context-dependent, high and low frequency contact calls (PADILLA DE LA TORRE et al., 2016). KILEY (1972), RUSHEN et al. (1999), WEARY E CHUA (2000), THOMAS et al. (2001), JOHNSEN et al. (2018) and GREEN et al. (2020) claimed that cattle vocalizations in response to calf separation and social isolation present increases in pitch, related to a more reactive or excited individual. Our results are consistent with the suggestion that higher call frequencies (Hz) are related to negative valence and to animals subjected to high intensity emotions (ZAVY et al. 1992; FAZIO et al. 2005), while low frequency in Hz tends to be associated with positive states (DOYLE e MORAN, 2015; EDE et al., 2019; PADILLA DE LA TORRE et al., 2016; WATTS E STOOKEY, 1999). In addition, based on PADILLA DE LA TORRE et al. (2015) and PADILLA DE LA TORRE e MCELLIGOTT

(2017), low pitch calls were produced by cows closed to their calves and high pitch calls were expressed by cows separated from their calves, without visual contact. High frequency calls play the biological role of long distance communication, indicate suffering and motivation to restore social contact, while low frequency calls are used for closed contact communication and as a harmonization mechanism (PADILLA DE LA TORRE et al., 2015; SIEBERT et al., 2011). Therefore, our findings provide additional evidence which may foster increased field use of this valuable welfare indicator. A potential practical improvement may be the training of livestock staff to recognize different call pitches and avoid handling procedures which are associated to negatively-valenced calls.

The negative valence in T2 was associated to higher number of vocal emissions in CHAR, LIMO and BRAH dams, probably with the emergency purpose of communication between the pairs and in an attempt to call for a reunion. Regarding breed differences, LAY et al. (1998) stated that in response to weaning, Brahman calves vocalized more and had higher average plasma concentrations of cortisol and heart rate as compared to the calves that remained with their mothers. Furthermore, DESTREZ et al. (2018) observed that Charolais animals vocalized less in positive situations as compared to negative practices. Further studies seem warranted to better understand the genetic influences on vocalization characteristics of cattle.

In our experiment, besides high pitch and longer vocal calls, behavioural changes were identified in T2, such as tail flapping, sniffing the corral, and head and body movements. An increase in the number of calls may be linked to stress in cattle (CANALI et al., 2001; MEEN et al., 2015; PROBST et al., 2014; WATTS e STOOKEY, 1999) and periods of social isolation (MÜLLER e SCHRADER, 2005; RUSHEN et al., 1999). Regarding behavioural signals, negative emotional states and stressful situations can stimulate more frequent tail movements when compared to control and positive stimuli groups (DOYLE e MORAN, 2015; LEE et al., 2009; SCHWARTZKOPF-GENSWEIN et al., 1997; TURNER et al., 2020). As for sniffing the corral, LE NEINDRE (1989) showed more frequent wall-sniffing when calves were separated from their mothers. Furthermore, according to JOHNSEN et al. (2015) and TURNER et al. (2020), frequency of head movement increased in cattle during isolation and stressful handling. Moreover, mother-calf separation is reported as a stressful experience for both and induces behavioural signs of distress and anxiety, associated with an increase in locomotor activity (FLOWER e WEARY, 2001; HALEY et al., 2005;

HUDSON e MULLORD, 1977; LEE et al., 2009; PRICE et al., 2003; SOLANO et al., 2007; VEISSIER et al., 1989; WEARY e CHUA, 2000). The fact that male calves showed more ear movements may be related to reports that they are significantly more active than females (REINHARDT et al., 1978), which in turn may be related to the development of male fighting skills and protection of the herd against predators (MATHISEN et al., 2003). Therefore, the behavioural signals found in our study are likely to indicate that T2 is an emotionally negative situation.

In our study, both cows and calves in response to separation had higher call counts and these calls decreases when the conspecifics were brought back. The reunion of the pair reduced behavioural suffering (BOISSY e LE NEINDRE, 1997) and the increase in the number of dam calls may be a sign that the cows responded maternally and searched for the calf (HOPSTER, 1998). For example, after the cow-calf reunion (T3) there was an increase in the mothers' approach to their offsprings. Similar situation and correspondent behavioural signs were reported before by HUDSON e MULLORD (1977) and TURNER et al. (2020), resulting in dams running to their offspring and an increase in the frequency of checking the calf. Also, dams may have sniffed their offspring after reunion with the purpose of further identifying them. In this sense, olfactory memory helps animals to recognize other individuals (JOHNSEN et al., 2016; LÉVY et al., 2004; MOUNAIX et al., 2014). Alternatively, the decrease in uneasiness and the reduction in the signs of distress of the pair after the reunion that we have observed is consistent with the calmer behavioural signs observed in the reunion of cows and calves in other studies (LEFCOURT e ELSASSER, 1995; SOLANO et al., 2007). Also, BOISSY e LE NEINDRE (1997) reported, in cattle, higher general activity during separation and decreased excitement levels with the reunion of the animals. Thus, both vocalization and other behaviour results in our study seem to suggest they are useful and coherent indicators to differentiate positively from negatively-valenced situations in cattle.

3.5 CONCLUSION

In this study, a series of indicators were identified that can be adopted as non-invasive tools to assist in the assessment of animal welfare and in the understanding of the emotional state of cattle in both potentially negative and positive situations. The indicators from cattle vocal responses that seem related to emotional valence are latency, duration, pitch and count of vocalization, and those from other behavioural responses are tail flapping, cow-calf approaching, sniffing the environment, cow-calf sniffing, and movement of the head and the ears. The negatively-valenced scenario resulted in higher tail flapping, higher ear, head and body movement, as well as sniffing the environment for cows and calves. On the other hand, the positive scenario outcomes were vocalization with shorter duration, lower pitch and vocalization counts, as well as less tail flapping in calves. Future studies to better understand the effects of sex, breed and parity number on the relevant indicators seem warranted.

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4 VOCALIZATION AND OTHER BEHAVIORS INDICATING PAIN IN BEEF CALVES DURING THE EAR TAGGING PROCEDURE

RESUMO

A vocalização e outros sinais de comportamento podem ser usados como ferramentas para avaliação do bem-estar animal no manejo de bezerros de corte. O objetivo deste trabalho foi comparar parâmetros vocais e sinais comportamentais expressos por bezerros de corte submetidos ao procedimento de marcação auricular para identificação (TE) e as respostas ao toque humano nas orelhas (TL). Participaram do estudo 52 bezerros taurinos de corte, 30 machos e 22 fêmeas, com 91,3 ± 28,1 dias de idade, em Santa Catarina, Sul do Brasil. Os bezerros foram divididos aleatoriamente em dois grupos (TE e ST) e registrados por um minuto para análise posterior. Mais animais do grupo TE vocalizaram durante o ensaio (14; 5), com maior número de vocalizações (1,7; 0,3), bem como maior número de movimentos da cabeça (7,8; 4,0), abanamento da cauda (56,1; 29,8) e movimentos das pernas (28,4; 16,4). As vocalizações masculinas foram mais longas do que as femininas (2,07 s; 1,61 s), com maior frequência fundamental (249,6 Hz; 178,6 Hz). Além disso, bezerros mais velhos vocalizaram com frequência fundamental mais alta (241,0 Hz; 212,8 Hz) e apresentaram mais movimentos da cabeça (6,5; 5,3) do que os mais jovens. Os resultados sugerem que as características de vocalização associadas a outros sinais de comportamento podem ser utilizadas como ferramentas para avaliar a dor em bezerros de corte durante procedimentos invasivos, como manuseio de identificação.

Palavras-chave: 1.Análise acústica; 2.Avaliação de emoções; 3. Bem-estar animal; 4.Comunicação vocal; 5.Parâmetros acústicos; 6.Sinais comportamentais.

ABSTRACT

Vocalizations and other behaviour signals can be used as tools to animal welfare assessment in beef calves handling. This paper aimed to compare vocal parameters and behaviour signals expressed by beef calves submitted to ear tagging procedure for identification (ET) and the responses of a human touch in the ears (ST). The study was carried out using a total of 52 taurine beef cattle calves, 30 male and 22 female, with average age of 91 days, in Santa Catarina, South Brazil. Calves were equally and randomly divided into two groups (ET and ST) and the records of the first 10 seconds of each animal was analysed. In comparison with ST, ET group had more animals that vocalized during the trial (14; 5), a higher number of vocal calls (1.666; 0.338), as well as a higher number of body movements: head movements (7.763; 3.961); tail flapping (56.066; 29.776); and legs movement (28.407; 16.379). Also, male vocalizations were longer than female (2.074 s; 1.609 s), and had higher fundamental frequency (249.642 Hz; 178.631 Hz). Additionally, older calves have higher fundamental frequency (240.998 Hz; 212.832 Hz) and more head movements (6.527; 5.305) than younger. The results suggest that the vocalization analysis associated with other behaviour signals could be used as tools to assess pain sensations in beef calves during identification handling.

Keywords: 1.Acoustic analysis; 2.Acoustic parameters; 3.Animal welfare; 4.Behavioral signs; 5.Emotion assessment; 6. Vocal communication

4.1 INTRODUCTION

The acknowledgment and assessment of the pain in farm animals involve ethics and welfare aspects. Good animal husbandry practices for livestock may be guided by the Five Freedoms defined by the Farm Animal Welfare Council (FAWC, 1993). According to this standard, animals must be free from pain, injury and disease by prevention or rapid diagnosis and treatment. The concept of pain was described by MERSKEY (1979) as an unpleasant sensorial and emotional experience. Thus, considering its physical and mental dimensions (ANIL et al., 2005), pain recognition and mitigation are important to improve animal welfare.

Pain provokes aversive reactions and has implications in health and welfare (DOYLE e MORAN, 2015). Also, it is associated with substantial suffering, which is the extension of one or more unpleasant feelings for more than few seconds or minutes; then, pain is particularly significant in animal welfare studies (BROOM e JOHNSON, 1993; BROOM, 2001). In addition, the recognition of pain is mandatory for its relief (GLEERUP et al., 2015). Therefore, behaviour studies are essential in translating and analysing expressions of emotional states (GONYOU, 1994), so that pain can be recognised in nonverbal individuals. Vocalization is reported as the most evident behaviour induced by pain or discomfort in cattle. Accordingly, it is used for comparison and determination of husbandry and livestock interventions (GRANDIN, 1998; GREEN et al., 2018). Pain and disease are key concepts for animal welfare according to public perception (MILLMAN, 2013).

WATTS e STOCKEY (2000) claimed that, in experimental conditions involving pain, the vocal responses bring information regarding animal state and that they are useful as welfare indicators. This is due to the changes in vocal characteristics according to the excitation or intensity of the emotional states (WATTS e STOOKEY, 1999; WEEKS, 2008; BRIEFER, 2012). The vocal calls in cattle are important for communication (PHILLIPS, 1993) and cattle can produce specific vocalization types, as well as abundant calls due to pain stimulation (SCHWARTZKOPF-GENSWEINS et al., 1998). It has been proposed that these calls may act as a warning for other herd members to avoid painful situations (DOYLE & MORAN, 2015).

The observation of vocalization is a non-invasive tool, cheaper and easier to use as compared to other pain measures, such as biochemical parameters (NDOU et al., 2011), which justifies its adoption as one of the indicators to assess animal welfare.

Even though acoustic analysis is an important evaluation in cattle (YAJUVENDRA et al., 2013), the interpretation of vocalization in isolation is not complete for welfare assessment (MANTEUFFEL et al., 2004). Thus, vocal call analysis may be associated with other behavior records to provide evidence of this particular mental state. Besides vocalization, sensation of acute pain includes vigorous and coordinated attempts to escape or to remove the source of intense stimulation (BATESON, 1991). In the case of cattle, unpleased situations such as pain can scare the animals, who may respond jumping, increasing locomotor activity and changing their posture (BROOM e JOHNSON, 1993; CURRAH et al., 2010), shaking their heads (SCHWARTZKOPF-GENSWEIN et al., 1998; STILWELL et al., 2010), moving their bodies (GRONDAHL-NIELSEN et al., 1999), and flapping their tails (PETRIE et al., 1995; DOYLE e MORAN, 2015).

As CLARK et al. (2016) study reported, people are concerned with farm animal treatment, and the quality and safety of the final product. Also, people declare that improvements are needed to animal husbandry. Regarding animal care, veterinarians and cattle producers also recognize the need for changes in painful procedures (SUNMER et al., 2018). There has been increasing public awareness of farm animal welfare issues and a concomitant increase in animal welfare research and teaching activities (MENCH et al., 2008).

According to OIE (2011), animal traceability is the ability to follow one or more animal during lifetime, which requires animal identification combined with their registration. In Brazil, the official system responsible for identification and certification of cattle was first specified in the Normative Instruction n°1, January 2002 (BRASIL, 2002), leading to the traceability service for the cattle chain, named SISBOV, established in July 2006 (BRASIL, 2006). This system fulfils the European Union requirements (REGATTIERI et al. 2007) and is mandatory for foreign trade as well as for foot-and-mouth disease virus free zones (OIE, 2007). In Santa Catarina, cattle herds are supervised by the Integrated Company of Agricultural Development (CIDASC) and animals are identified by a numbered ear tag (CIDASC, 2018). Ear tagging is an invasive procedure that causes pain (LOMAX et al., 2017), increases the number of vocal calls and head movements, and may cause local inflammatory reaction (EDWARDS et al., 2001). Thus, it reduces welfare (CAI e LI, 2013). According to ADCOCK e TUCKER (2017), animal identification with hot or cold iron induces immediate pain responses, such as tail flapping and head movements. However, the same study reported the need for studies to evaluate other identification methods, like ear tagging and electronic methods with potential to reduce pain. The aim of this work was to study vocal parameters and behavioural signs showed by cattle during ear tagging, to advance knowledge on pain recognition in bovine individuals and contribute to the understanding of potential on-field indicators.

4.2 MATERIALS AND METHODS

4.2.1 Animals

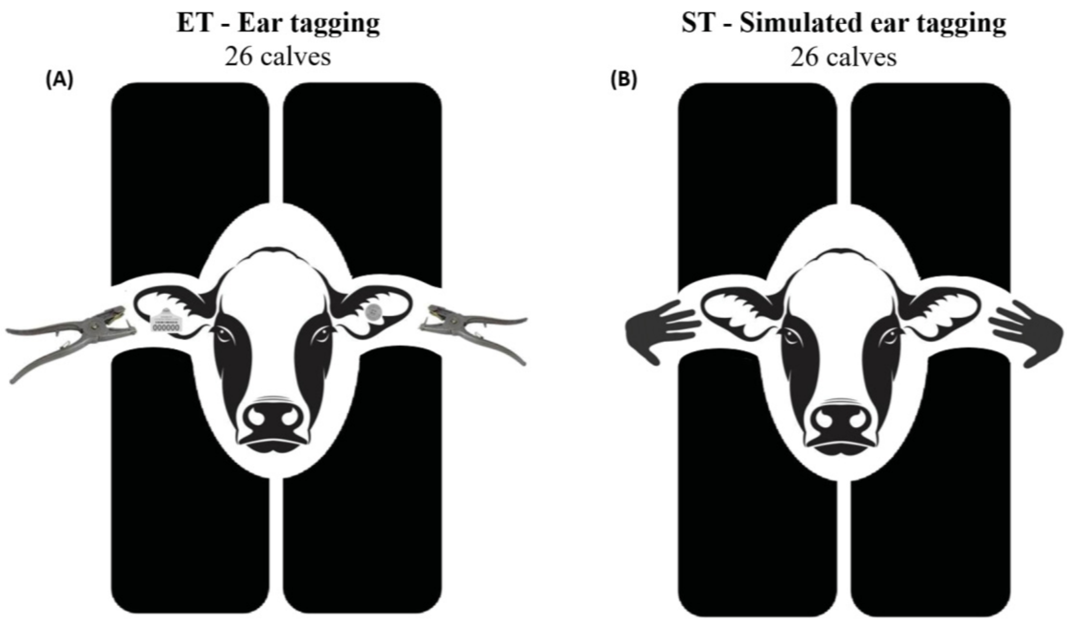
A total of 52 taurine beef cattle calves, 30 male and 22 female, 91.3 ± 28.1 d-old, participated in this study. Ear tagging procedures in all animals were video-recorded for further analysis. The experiment was conducted in Canoinhas, Santa Catarina, South Brazil, at Fazenda Nova Esperança (20°09'40, 25” S; 50°32'39,78” O), in August 2018.

4.2.2 Inductive management vocal responses and body posture reactions

Selected behaviour signals were analysed during ear tagging, which is a mandatory procedure for individual animal identification and registration within beef cattle herds in the State of Santa Catarina, Brazil. We used concepts of SCHWARTZKOPF-GENSWEIN et al. (1998) and COETZEE et al. (2008) to compare the responses caused by painful and unpainful procedures in cattle. The 52 calves were randomly divided in two groups: ear tagging (ET), composed by 26 calves who experienced the painful procedure of tagging each ear with an auricular button of registration; and simulated ear tagging (ST), composed by the other 26 animals who were not effectively tagged but experienced a human touch on their ears for 10 seconds, which is the time required for the tagging procedure (FIGURE 6). All animals were handled similarly for the entry to the cattle head gates.

The vocalizations and other behavioural signals were recorded by two digital cameras (Sony Cyber Shot DSC-W610 14.1 Megapixels) for later analysis of the audios and footage at the laboratory. The cameras were placed on tripods and were turned on before the animals arrived at the gates. A camera was installed in front of the animals, to record vocal calls and head movements, and a second camera was installed on the side of the animals, to record other body reactions. The recordings lasted 1 min after the beginning of the stimulus for both ET and ST situations.

FIGURE 6 - REPRESENTATION OF THE EXPERIMENTAL DESIGN: (A) EAR TAGGING (ET) WITH AN AURICULAR BUTTON OF IDENTIFICATION IN EACH EAR; (B) SIMULATED EAR TAGGING (ST) WITH A HUMAN TOUCH ON THE EARS FOR 10 SECONDS.



4.2.3 Behavioural signals

The audacity software (version 2.1.3) was used to extract the audio from the video recordings. The acoustic signals were sampled at a rate of 44.1 kHz, quantized to 16 bits and stored in .wav format files on a MacBook Pro computer. Then, using the software Praat (version 5.3.55), which is a free software developed by Paul Boersma and David Weenink (2013), at the Center for Phonetic Sciences at the University of Amsterdam. The acoustic signals were analysed according to studies of GREEN et al., (2019), KIM et al. (2019), VOLODIN et al. (2017) and YAJUVENDRA et al. (2013).

The vocalization variables and other behaviour signals were evaluated separately for each individual, for the total duration of the 1 min recordings. The acoustic parameters selected to be analysed were the number of calves that vocalized in each group (LAY JR et al., 1992; GRANDIN, 1998); the number of vocal calls per animal (SCHWARTZKOPF-GENSWEIN et al. 1998; DOHERTY et al., 2007; CURRAH et al., 2009) represented by the total vocal calls emitted by one individual during the recordings; the fundamental frequency (f0) (WATTS e STOOKEY, 1999), intensity

(GREGORY et al.,2007; WEEKS et al., 2009; IULIETTO et al., 2018) and the duration of each vocal call (RUST et al., 2007).

The behaviour signals studied were head shaking (STILWELL et al., 2010), which is the sum of every time the head was moved up, down or to the side; tail flapping (DOYLE e MORAN, 2015), measured as each movement of the tail away from the body until the return to the relaxed position; and body movements (GRONDAHL-NIELSEN et al., 1999), including kicks, leg lifting as well as leg movements to the front, back or one of the sides.

4.2.4 Statistical analysis

The group effect on behaviour and vocalization variables was assessed using regression models in two moments: adjusted and non-adjusted by covariates. For counting variables, related to animal behaviour and number of vocalizations, we adjusted the group effect by age and sex, while for the remaining vocalization variables, we also included the number of vocalizations as an additional covariate. The model parameters were estimated using a quasi-likelihood approach, and robust standard errors were obtained to avoid possible model misspecifications (YAN e FINE, 2004). The results are presented by means of estimated marginal means and corresponding 95% confidence intervals. The conclusions are based on a significance level of 5%. All analyses were performed in R statistical environment, version 4.0.2. (TEAM, 2020). The geepack library was used to fit the regression models (HALEKOH et al., 2006), whereas the marginal means were calculated using the emmeans library (LENTH, 2020).

4.2.5 Ethical statement

This study was performed in accordance to Law 11,794, of October 8, 2008, Decree 6,899, of July 15, 2009, and the directions of the National Council for the Control of Animal Experimentation (CONCEA). This study was approved by the Ethics Committee on the Use of Animals (CEUA) of the Agricultural Sciences Sector of the Federal University of Paraná, Brazil, with degree one of invasiveness, at a meeting on 06/02/2017, certified by protocol number 048/2017.

4.3 RESULTS

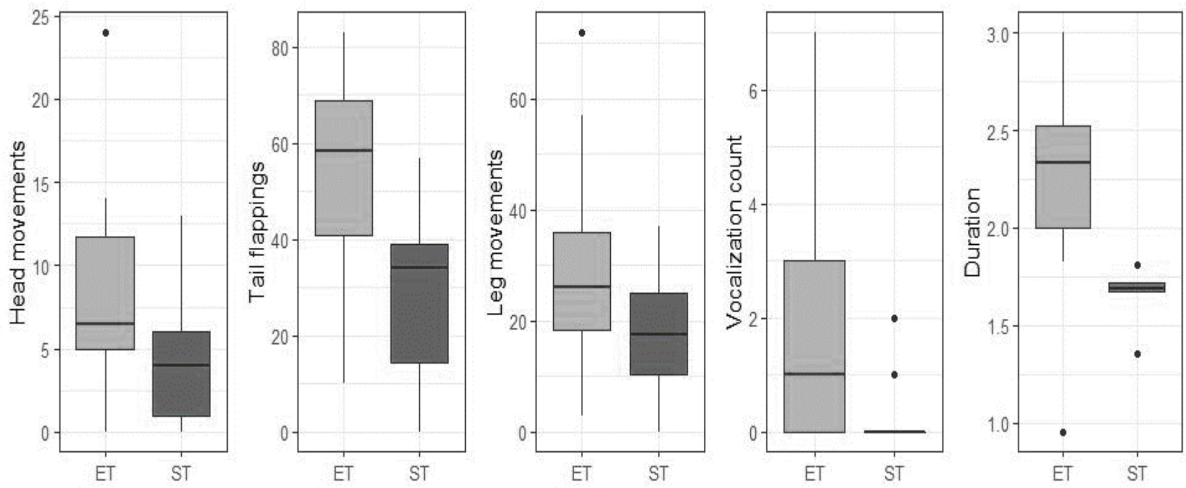
The ET group presented a higher number of calves that vocalized than the ST group (14/26; 5/26), which resulted in 4.74 higher chance of vocalization in ET (IC 95%; 1.23; 21.29) in comparison with ST (P-value= 0.02). As for the number of vocalizations in each group, ET animals vocalized 4.9 times more (P-value= 0.001) with vocal calls 60.8% longer than ST (P-value<0.001). Also, the ET group presented more head, tail and leg movement than ST. Regarding body movements, animals in ET moved their heads 95.9%, flapped their tails 88.3% and moved their legs 73.4% more often than those in ST (P-value<0.001) (TABLE 9) (FIGURE 7). Also, male calves emitted vocal calls 28.9% longer, with 38.8% times higher fundamental frequency than females (P=value<0.017) (TABLE 9).

TABLE 9 - MEAN AND CONFIDENCE INTERVAL (IC) OF VOCAL AND BEHAVIOUR VARIABLES, CONSIDERING THE EFFECTS OF EAR TAGGING (ET) AND SIMULATED EAR TAGGING (ST) GROUPS; SEX, MALE AND FEMALE; AND AGE, 86 DAYS (Q1) AND 111 DAYS (Q3).

* Different letters in the lines indicate significant differences within the categories (*P-value*<0.05). *Number of vocalizations per minute.

Variables		Group		Sex		Age	
		ET	**ST**	**F**	**M**	**86 (Q1)**	**111 (Q3)**
Vocalization count, frequency*	Mean	1.7^{A}	0.3^{B}	0.7^{A}	0.8^{A}	0.8^{A}	0.7^{A}
	CI (95%)	(1.0; 2.7)	(0.1; 0.8)	(0.3; 1.4)	(0.5; 1.4)	(0.5; 1.2)	(0.4; 1.2)
Duration, s	Mean	2.27^{A}	1.41^{B}	1.61^{A}	2.07^{B}	1.84^{A}	1.89^{A}
	CI (95%)	(2.09; 2.46)	(1.13; 1.70)	(1.27; 1.95)	(1.90; 2.25)	(1.65; 2.03)	(1.48; 1.98)
Fundamental frequency, Hz	Mean	208.3^{A}	219.9^{A}	178.6^{A}	249.6^{B}	212.8^{A}	241.0^{B}
	CI (95%)	(181.0; 235.6)	(186.9; 253.0)	(138.8; 218.4)	(218.3; 281.0)	(190.1; 235.5)	(212.2; 269.8)
Intensity, dB	Mean	79.8^{A}	80.2^{A}	79.5^{A}	80.6^{A}	80.0^{A}	80.3^{A}
	CI (95%)	(78.5; 81.1)	(79.1; 81.3)	(77.3; 81.7)	(80.1; 81.0)	(78.9; 81.1)	(79.4; 81.2)
Head movement, frequency	Mean	7.8^{A}	4.0^{B}	5.1^{A}	6.1^{A}	5.3^{A}	6.5^{B}
	CI (95%)	(6.3; 9.6)	(2.8; 5.6)	(3.7; 6.9)	(4.3; 6.6)	(4.3; 6.6)	(5.2; 8.1)
Tail flapping, frequency	Mean	56.1^{A}	29.8^{B}	41.2^{A}	40.6^{A}	41.2^{A}	39.6^{A}
	CI (95%)	(49.7; 63.2)	(24.3; 36.5)	(34.9; 48.5)	(35.2; 46.8)	(36.6; 46.4)	(34.7; 45.3)
Leg movements, frequency	Mean	28.4^{A}	16.4^{B}	19.5^{A}	23.8^{A}	21.4^{A}	22.3^{A}
	CI (95%)	(23.4; 34.5)	(12.9; 20.7)	(14.8; 25.8)	(20.4; 27.8)	(18.1; 25.2)	(19.1; 26.1)

FIGURE 7 - BOX PLOTS FOR HEAD MOVEMENT, TAIL FLAPPING, LEGS MOVEMENT, NUMBER OF VOCAL CALLS AND DURATION (S) IN EAR TAGGING (ET) AND SIMULATED EAR TAGGING (ST) GROUPS OF 26 CALVES EACH.



In relation to age, marginal means for 86 and 111 days were presented due to their first and third quartiles samples, respectively. Older calves moved 23% more their heads (P-value=0.024) and vocalized with 13.2% higher fundamental frequency (P-value=0004) than younger (Table 1). Finally, calves that vocalized less times (CI 95%; 1.7; 2.1) did so with 10.8% longer duration for their vocal calls (CI 95%; 1.48; 1.98) than animals with higher number of vocalizations (P-value=0.028).

4.4 DISCUSSION

Behavioural changes such as a higher number of animals vocalizing, higher frequency of vocal calls and locomotor activity are indicatives of pain (BROOM e JOHNSON, 1993; SCHWARTZKOPF-GENSWEIN et al., 1998). Coherently, the ET group had a higher number of calves vocalizing than ST, as well as more vocal calls. Vocalizations are one of the main behavioural signals of pain in cattle (GREEN et al., 2018); thus, the number of vocalizations is higher in painful than in painless situations (WATTS e STOOKEY, 1999). Vocal emissions may be involuntary in relation to a painful state (WATTS e STOOKEY, 2000) or intentional as a warning signal to other members of the herd against a painful situation (DOYLE e MORAN, 2015). In the ET group, our results support the understanding of ear tagging as a painful procedure able to stimulate the peripheral and central nervous systems, which are directly associated with the perception of pain (YAM et al., 2020). Also, the vocalizations in this group may be a form of communication, warning other animals, especially members of the group, about a painful experience.

The ET group had longer vocal calls during the experiment. Unpleasant situations such as pain may cause longer vocalizations because they tend to lead to excitement, increasing action and tension in respiratory muscles, which may be related with longer durations. Also, a sense of urgency seems to cause extended calls (BRIEFER, 2012). In addition, as cattle use vocalization to inform co-specifics (WEEKS, 2008), longer calls may promote a more effective communication with the herd regarding the painful situations, as well as improve the request for help. Thus, the longer calls observed in ET animals are aligned with present knowledge regarding reactions to pain in cattle.

According to GRONDAHL-NIELSEN et al. (1999) and SCHWARTZKOPF-GENSWEIN et al. (1998), painful stimuli are responsible for more head shaking in comparison to control animals, which is confirmed by our results, as the ET group presented more head movements than the ST group. Overall, head movement scales may be used as an indicator of pain, and higher number of head movements is a sign of disturbance as animals attempt to turn their heads towards the stimulus (GIGLIUTO et al., 2014). Moreover, regarding ear tagging, the increase in head movements may be an attempt to escape the painful stimulus and additional tissue damage (MOLONY e KENT, 1997).

As expected, the ET group presented more tail flapping than the ST animals. The tail is an important element for behavioural signs in cattle. Tail flapping is common when animals are agitated and nervous due to a threat or painful stimulus, which in general increases their movements (DOYLE e MORAN, 2015). Accordingly, SYLVESTER et al. (2004) trial showed more tail flapping in calves that experienced a painful procedure in their heads in comparison to the control group. Thus, pain seems to be translated to more tail movements and this may relate to a necessity of communicating the experience to others. Also, according to GRANDIN et al. (2014), higher frequency of tail flapping can precede more violent reactions, such as kicks or attempts to scape.

Moreover, the ET group showed more leg movements in comparison to ST. These results may be due to protective behaviour and attempts to escape the painful stimulus. The increase in locomotor activity, including restlessness and leg movements, is known to be related to pain (MOLONY e KENT, 1997). The leg movement results have added to other behavioural signals in indicating that the typical ear tagging procedure induces pain in cattle.

Our results showed different f0 in relation to sex: male calves had higher f0 than female. The f0 of vocal calls tends to increase as cattle are more excited or agitated (DOYLE e MORAN, 2015). Male calves seemed more reactive to the painful stimulus and to the head gate, as per field observation. As these variables were not formally assessed, this difference in f0 warrants further studies. In addition, older calves presented higher f0 and more head movements as compared to younger ones. According to MOLONY et al. (1993) and THORNTON e WATERMAN-PEARSON (2002), older animals present more sensibility and facility to express pain. These factors may relate to the higher f0 we observed in older in comparison to younger animals. Furthermore, f0 in cattle can rise due to an increase in excitement, which may be related to aversive situations (PADILLA DE LA TORRE et al., 2015; GREEN et al., 2018). Finally, calves that vocalized less frequently emitted longer calls, which is related to the higher number of vocalizations in the same time period as that measured for shorter vocal calls.

Overall, our study confirmed that ear tagging is a painful method for animal identification and there is an urgent need for research on alternative and painless methods for individual animal identification. In addition, it is important to note that analgesia diminishes the behaviour signals related to pain (GRONDAHL-NIELSEN et al., 1999; STAFFORD e MELLOR, 2011), which is then a relevant practice to be included in painful invasive procedures, such as the identification of cattle through ear tagging.

4.5 CONCLUSION

This study suggests that the analysis of vocalization, including the number of animals vocalizing, the number and duration of vocal calls, as well as other behaviour signals such as head, tail and leg movements, may be used as indicators for assessing pain in beef calves during the identification management. The results showed more calls and body movements in the group of calves that had their ears effectively tagged. Also, male calves emitted vocal calls longer and with higher F0 than females. Additionally, older calves presented more head movements and higher F0 than younger. Thus, although procedures such as ear tagging may be mandatory, it is important to consider painless alternatives for the individual identification of animals or effective forms of pain control during all invasive procedures, especially those which are systematic and, thus, allow for adequate planning.

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5 FINAL CONSIDERATIONS

This thesis contributed to identify vocal indicators related to emotional valence such as number of vocalizations emitted, latency, duration and fundamental frequency. Additionally, it recognized other behavioral signs dependent on the emotional state, such as tail flapping, head, ear, leg and body movements, frequency that cow approached calf, cow and calf sniffed corral and cow sniffed calf. The findings of this research show that the vocal responses emitted and the body signals in situations of opposite emotional valence carry different information and peculiar characteristics. It was also possible to verify that the separation of cows and calves is a negative stimulus that causes stress, as well as the confinement of cats in transport boxes and the ear tagging procedure in cattle. On the other hand, the meeting of the calf with its mother is a positive situation that causes positive emotional states, as well as the offering of favorite snacks to cats. Thus, in different species, cattle and cats, similar presentations of these indicators were found in situations of the same emotional value, which leads us to think that it is possible to extrapolate these parameters to other species of mammals, and strengthens the claim that vocalization it is strongly related to the emotions experienced by animals, in addition the vocal indicators also seem capable of transmitting information related to sex, age and genetic ancestry.

When analysing different scenarios, positive and negative, it was found that the vocal parameters can be used as indicators and non-invasive methodologies to help in the analysis of animal welfare, from the recordings of the so-called vocal and analysis of its variables. Also, this study suggested that vocalizations may be an efficient and feasible tool to assess animal welfare due to its practical, repeatable and valid characteristics. Animal welfare conceptualization and awareness has been increasing in the last years. Thus, it is fundamental to elaborate correlations of distinct species and their physiological, behavioural and emotional aspects to develop applicable protocols for routine assessment of animal welfare.

Monitoring the vocal calls of cats may be valuable to improve human-animal relationships in the sense of understanding their needs and minimizing their exposure to aversive situations. This experiment suggested the correlation between coat color and vocalization, which may be associated with the evolution of domesticated cat characteristics and temperament. Future studies are needed for this regard. Also, it would be interesting to analyse morphological characteristics and cat's vocal repertoire, besides the meowing.

The number of vocalizations is already observed in official protocols, such as the Welfare Quality, to assess pain and fear; however, it is necessary to have a robust evaluation of vocalization rather than only counting vocal calls. Vocalization assessment in mandatory handling procedures for beef cattle may be relevant to recognize pain and discomfort. Also, these vocal parameters are valuable to identify critical procedures that promote unnecessary pain for farm animals, such as the traditional ear tagging in calves. In addition, its applicability is important to improve the welfare in herd management, such as minimizing the separation of calves and cows.

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ANNEX 1 – SUBMISSION OF ARTICLE INTITLED "CAT VOCALIZATION IN AVERSIVE AND PLEASANT SITUATIONS"

*This is an automated message. *

Journal: Journal of Veterinary Behavior
Title: Cat vocalization in aversive and pleasant situations
Corresponding Author: Miss Marina Heidemann
Co-Authors: Maria Alice Schnaider; Adelaide Silva; Cesar Taconeli; Carla Molento
Manuscript Number:

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Journal of Veterinary Behavior

ANNEX 2 – PUBLISHED ARTICLE INTITLED "ONLY WHEN IT FEELS GOOD: SPECIFIC CAT VOCALIZATIONS OTHER THAN MEOWING"

animals

MDPI

Article

Only When It Feels Good: Specific Cat Vocalizations Other Than Meowing

Jaciana Luzia Fermo [1], Maria Alice Schnaider [1], Adelaide Hercília Pescatori Silva [2] and Carla Forte Maiolino Molento [1,*]

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Received: 31 August 2019; Accepted: 14 October 2019; Published: 29 October 2019

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Simple Summary: Among carnivore animals, domestic cats are those with the most extensive vocal repertoire. This is due to their social organization, nocturnal activity and long period of contact between the mother and the offspring. In order to identify vocalizations other than meowing in two different situations, a study was performed with 74 cats divided into two groups, one associated with a pleasant situation and another with an aversive situation. Only the group exposed to the positive stimulus of being offered a favorite snack produced specific vocalizations other than meowing: recognition or trill, squeak, purring and chatter. During the aversive situation of car transport, no vocalization other than meowing was observed. The present study indicates the relevance of applying the study of vocalizations to determine the state of emotional valence in cats.

Abstract: Our objective was to identify and characterize the types of vocalization other than meowing (VOM) in two contexts, a pleasant and an aversive situation, and to study the effect of the sex of the animal. A total of 74 cats (32 tom cats and 42 queens) living in the city of Curitiba, Brazil, participated in the study; in total, 68 (29 tom cats and 39 queens) were divided into two groups according to the stimulus they were exposed to: either a pleasant situation (PS), when they were offered a snack, or an aversive situation (AS), with the simulation of a car transport event. The other six animals (three tom cats and three queens) participated in both situations. Only the PS group presented VOM; of the 40 PS animals, 14 presented VOM, mostly acknowledgment or trill and squeak. No correlation was observed between vocalization and cat sex ($p = 0.08$; Pearson's Chi-Square). Results show that VOM is exclusively associated with positive situations, suggesting that these vocalizations may be relevant for understanding the valence of cat emotional state. Further studies are warranted to advance knowledge on other VOMs and on the generalization of our findings to other situations.

Keywords: cat behavior; *Felis catus*; phonetics; welfare

ANNEX 3 – SUBMISSION OF ARTICLE INTITLED "VOCALIZATION AS AN INDICATOR OF EMOTIONAL VALENCE: THE CASE OF COW-CALF SEPARATION AND REUNION"

*This is an automated message. *

Journal: Journal of Veterinary Behavior
Title: Vocalization as an indicator of emotional valence: the case of cow-calf separation and reunion
Corresponding Author: Miss Marina Heidemann
Co-Authors: Maria Alice Schnaider; Adelaide Silva; Cesar Taconeli; Carla Molento
Manuscript Number:

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Journal of Veterinary Behavior

ANNEX 4 – SUBMISSION OF ARTICLE INTITLED “VOCALIZATION AND OTHER BEHAVIORS INDICATING PAIN IN BEEF CALVES DURING THE EAR TAGGING PROCEDURE”

*This is an automated message. *

Journal: Journal of Veterinary Behavior
Title: Vocalization and other behaviors indicating pain in beef calves during the ear tagging procedure
Corresponding Author: Miss Marina Heidemann
Co-Authors: Maria Alice Schnaider; Adelaide Silva; Cesar Taconeli; Carla Molento
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Journal of Veterinary Behavior

ANNEX 5 – PUBLISHED ARTICLE INTITLED "COMPASSIONATE CONSERVATION: CONCEPT AND APPLICATIONS"

Archives of Veterinary Science **ISSN 1517-784X**
v.22, n.4, p.116-130, 2017 www.ser.ufpr.br/veterinary

COMPASSIONATE CONSERVATION: CONCEPT AND APPLICATIONS

(Conservação compassiva: conceito e aplicações)

Arendy Ribeiro Villa Branco[1], Vanessa Souza Soriano[2], Maria Alice Schnaider[2], Carla Forte Maiolino Molento[2*]

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RESUMO: O crescimento das áreas urbanas, a falta de familiaridade das pessoas com a vida selvagem, a disseminação das cidades para regiões não exploradas e a perda de habitats naturais fazem com que animais e seres humanos vivam em grandes proximidades dentro e ao redor de áreas urbanas. Esta situação impõe muitos desafios a ambas as partes e ocasionalmente gera conflitos. Ao gerenciar situações de conflito, a abordagem tradicional considera quase exclusivamente a conservação, com pouca ou nenhuma consideração pelo bem-estar animal. Este trabalho tem por objetivo discutir o conceito de conservação compassiva e sua aplicação prática pela exposição de três questões relevantes de conservação de espécies silvestres. A abordagem de conservação compassiva une a conservação e o bem-estar animal em um esforço para tomadas de decisão mais esclarecidas e, consequentemente, melhoria do bem-estar animal e humano e de aspectos ambientais. Em uma revisão sobre alguns métodos de manejo da vida selvagem, as abordagens são comparadas quanto a pontos fortes e fracos em relação ao bem-estar e à conservação animal.

Palavras-chave: Animais selvagens; armadilhas de cola; bem-estar animal; bem-estar único; capivara; urso.

ABSTRACT: The growth of urban areas, the unfamiliarity of people with wildlife, the spread of cities into wilderness and the loss of natural habitats cause animals and humans to live in close proximity in and around urban areas. This situation imposes many challenges to both parties and occasionally generates conflicts. When managing conflict situations, the traditional approach considers almost exclusively conservation, with little or no regard for animal welfare. This work aimed to discuss the concept of compassionate conservation and its application in practice by addressing three relevant wildlife conservation issues. The compassionate conservation approach unites conservation and animal welfare in an effort for more enlightened decision making and, consequently, improvement in animal and human welfare and environment aspects. In an overview of some wildlife management methods, the approaches are compared regarding strengths and weaknesses on animal welfare and conservation.

Key Words: Animal welfare; bear; capybara; glue traps; one welfare; wildlife.

ANNEX 6 – PUBLISHED ARTICLE INTITLED "DEVELOPMENT AND REFINEMENT OF THREE ANIMAL-BASED BROILER CHICKEN WELFARE INDICATORS"

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Hertfordshire AL4 8AN, UK
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Animal Welfare 2018, 27: 263-274
ISSN 0962-7286
doi: 10.7120/09627286.27.3.263

Development and refinement of three animal-based broiler chicken welfare indicators

APO Souza[†], VS Soriano[†], MA Schnaider[†], DS Rucinque[‡] and CFM Molento[*†]

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Abstract

*This study aimed to refine bird-soiling as a broiler chicken (*Gallus gallus domesticus*) welfare indicator, and to develop and test two additional indicators, namely contact dermatitis on the breast and abdominal areas and carcase scratches. We constructed a questionnaire with pictures of birds presenting different indicator levels for classification as absent, low, moderate or severe. The questionnaire was sent to 146 invited experts for the first round and 88 for the second, in a Delphi process. Visual scales were built for the target indicators, which were tested by three assessors in ten flocks on-farm (n = 1,303 birds) and at the slaughterhouse (n = 1,631 birds). High concordance was observed among groups of Delphi respondents and among assessors. A total of 90.7% of the birds were either moderately or severely soiled, 99.9% were poorly feathered, 73.4 and 90.0% presented erythaema and carcase scratches, respectively. The correlations between litter quality and all outcomes assessed on-farm, and between bird-soiling and contact dermatitis on the breast and abdominal areas, were moderate. Results suggest that adoption of the proposed scales may improve our ability to assess broiler chicken welfare, since relevant problems were prevalent and measurement consistency acceptable. Substantial concordance observed among assessors encourages application of these animal-based indicators to assess broiler chicken welfare in a wide range of poultry houses, in a variety of different countries, thereby allowing the scales to be tested in a host of animal welfare conditions.*

Keywords: *animal welfare, outcomes, plumage cleanliness, skin irritation, welfare assessment, welfare measures*

ANNEX 7 – PUBLISHED ARTICLE INTITLED "ATTITUDE OF BRAZILIAN CONSUMERS ON ANIMAL WELFARE"

REVISTA ACADÊMICA: CIÊNCIA ANIMAL

OPEN ACCESS

ARTIGO ORIGINAL

Atitude de consumidores brasileiros sobre o bem-estar animal

Attitude of Brazilian consumers on animal welfare

Bruna Maria Remonato Franco, Elaine Cristina de Oliveira Sans*, Maria Alice Schnaider, Vanessa Souza Soriano, Carla Forte Maiolino Molento

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Resumo

Os objetivos deste trabalho foram estudar e identificar as atitudes dos consumidores de produtos de origem animal com ênfase no bem-estar animal (BEA), possíveis vantagens do consumo de alimentos oriundos de melhores práticas de bem-estar e fatores que possam influenciar as decisões de compra. Para isto, foi realizada uma pesquisa online com 402 consumidores de produtos de origem animal provenientes das cinco regiões brasileiras. Os respondentes afirmaram que o tema BEA recebe pouca importância no país, avaliando as condições dos animais de produção no Brasil como piores quando comparadas às de outras nações, e atribuíram aos produtores rurais a principal responsabilidade quanto ao assunto. Os respondentes demonstraram uma desassociação entre o alimento e o animal que o originou, porém reconheceram que há influência na promoção do BEA ao adquirirem produtos diferenciados para tal atributo. Do ponto de vista dos respondentes, a disponibilidade de produtos com maior grau de bem-estar, e as informações disponíveis na rotulagem quanto à forma com que os animais são criados, constituem gargalos para a aquisição de tais produtos. O preço pode ser considerado um fator importante, visto que a maioria dos respondentes aceitaria pagar 10% (32,3%) ou 25% (24,6%) a mais em produtos produzidos com maior grau de bem-estar. Com base nos resultados, os respondentes brasileiros se importam com o BEA, porém, diversos fatores podem ser entraves para que exerçam sua preferência ética no comportamento de compra, como falta de informação para o consumidor antes e no momento da compra, baixa disponibilidade dos produtos, desassociação do produto com o animal de origem e custo elevado.

Palavras-chave: Animais de produção. Demanda. Disponibilidade. Mercado consumidor. Produtos de origem animal.

Abstract

The objectives of this study were to study and to identify consumer attitudes regarding animal welfare, possible advantages of food consumption from better welfare practices, and factors that may influence purchasing decisions. For this purpose, an online research with 402 consumers from five Brazilian region was conducted.

Edição Especial - Bem-estar e Comportamento Animal
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Recebido: 6 mar 2018 | Aprovado: 28 mai 2018

Rev. Acad. Ciênc. Anim. 2018;16 (Ed Esp 1):e161001
DOI: 10.7213/1981-4178.2018.161001
eISSN: 1981- 4178

ANNEX 8 – PUBLISHED ARTICLE INTITLED "PERCEPTION OF BEEF CATTLE PRODUCERS IN THE STATE OF PARANÁ REGARDING ANIMAL IDENTIFICATION BY HOT IRON BRANDING"

REVISTA ACADÊMICA: CIÊNCIA ANIMAL   OPEN ACCESS

ORIGINAL ARTICLE

Perception of beef cattle producers in the state of Paraná regarding animal identification by hot iron branding

Percepção de produtores de bovinos de corte do Paraná em relação à identificação animal a ferro quente

Bruno Roberto Müller*, Vanessa Souza Soriano, Elaine Cristina de Oliveira Sans, Maria Alice Schnaider, Carla Forte Maiolino Molento

LABEA - Animal Welfare Laboratory, Universidade Federal do Paraná (UFPR), Curitiba, PR, Brazil

Abstract

The aim of this work was to study the perception of beef cattle producers in the state of Paraná, Brazil, about hot iron branding. Seventeen beef cattle producers answered a questionnaire about their perspective on cattle identification methods and animal welfare aspects. Results showed that there is a consensus among farmers that the identification of animals at their farms is an important practice. The majority of farmers (12/17) use hot iron branding as the main method of identification of cattle and most farmers (11/17) believe it is an efficient method. Considering costs and applicability, 10/17 farmers believe there are other methods of identification that would be viable for utilization at their farms; ear tagging (7/17) and microchipping (3/17) were the most mentioned alternatives. Farmers affirmed believing that animals are sentient beings (16/17) and capable of experiencing pain (17/17). On a scale from 1-5, scores attributed to pain experienced capabilities of different species were high for human babies, sheep and dogs (median = 5.0). The median score attributed to the pain experienced by cattle during branding with a hot iron was 4.0, ranging from 2.0 to 5.0. In conclusion, the opinion of cattle producers in the State of Paraná, Brazil, indicates recognition of animal sentience and their ability to experience pain. Future efforts should focus on refining and developing new methods that are effective and inexpensive, motivating producers to use procedures that respect the quality of life of their animals.

Keywords: Bovine. Farmers. Identification. Opinion. Pain.

Resumo

O objetivo deste trabalho foi estudar a percepção de produtores de bovinos de corte do Paraná sobre a marcação de animais a ferro quente. Dezessete produtores de gado de corte responderam um questionário sobre sua percepção acerca da identificação de bovinos e sobre aspectos de bem-estar animal. Os resultados mostraram consenso sobre a importância da identificação dos animais em suas propriedades. A maioria dos produtores (12/17) usa o ferro quente como principal método de identificação do gado e julga que esta seja uma prática eficiente (11/17). Considerando custos e praticidade, 10/17 produtores

Special Edition - Animal Welfare and Behavior
* Corresponding author: brunormuller@yahoo.com.br
Received: 2 mar 2018 | Approved: 28 mai 2018

Rev. Acad. Ciênc. Anim. 2018;16 (Ed Esp 1):e161002
DOI: 10.7213/1981-4178.2018.161002
eISSN: 1981- 4178

ANNEX 9 – PUBLISHED ARTICLE INTITLED "CONSEQUENCES OF ARTIFICIAL SELECTION TO ANIMAL WELFARE"

REVISTA ACADÊMICA: CIÊNCIA ANIMAL OPEN ACCESS

ARTIGO DE REVISÃO

Consequências da seleção artificial para o bem-estar animal

Consequences of artificial selection to animal welfare

Elaine Cristina de Oliveira Sans [1], Larissa Helena Ersching Rüncos [1], Vanessa Souza Soriano [1*], Maria Alice Schnaider [1], Carla Forte Maiolino Molento [1]

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Resumo

A domesticação causou impacto importante na vida do ser humano e dos animais. Ao longo do tempo, foram descobertas novas formas de fazer com que os animais atendessem os interesses humanos, e uma das ferramentas utilizadas para alcançar tal objetivo foi a seleção artificial. O objetivo deste trabalho foi apresentar as consequências da seleção artificial para o bem-estar de animais de companhia e de produção. Embora os programas de seleção tenham importância econômica, os animais passaram a apresentar diversos problemas devido à intensificação do uso dessa ferramenta. Cães e gatos selecionados para características estéticas e animais de produção para características de desempenho sofreram deterioração crescente de seu bem-estar. A partir do reconhecimendo do impacto da seleção artificial sobre a qualidade de vida dos animais, é possível estimular a inclusão de aspectos relacionados ao bem-estar animal nos programas de seleção artificial para as diversas espécies que são utilizadas pelo ser humano.

Palavras-chave: Animais de companhia. Animais de produção. Genética. Sofrimento.

Abstract

Domestication had a great impact on both humans and animals. Over the time, new ways for making animals efficiently attend human needs have been discovered. One of the tools used to achieve this goal was artificial selection. The purpose of this work is to present the artificial selection consequences on animal welfare, including companion and farm animals. Even though selection programs have economic importance, animals have started to present several problems due to the intensification of the use of this tool. The proportion of dogs and cats selected for beauty characteristics and farm animals for performance has increased as well as the deterioration of their welfare. Once we can recognize the impact of artificial selection on the quality of animal lives, it becomes possible to stimulate the development of animal welfare items within the artificial selection programs imposed to the species used by humans.

Keywords: *Companion animals. Farm animals. Genetic. Suffering.*

Edição Especial - Bem-estar e Comportamento Animal

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Recebido: 12 abr 2018 | Aprovado: 1 ago 2018

Rev. Acad. Ciênc. Anim. 2018;16 (Ed Esp 1):e161110
DOI: 10.7213/1981-4178.2018.161110
eISSN: 1981- 4178