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Rogério Ribeiro Vicentini

Gyr cows (*Bos taurus indicus*) in the peripartum period: Assessment of calving prediction devices and factors affecting the maternal behavior and defensiveness

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Orientadora: Dra. Aline Cristina Sant'Anna
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We dream. We believe. We did. We won.

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“They say don't look back, but sometimes it's important to see how far you've come.”

– Unknown

RESUMO

O objetivo desta tese foi avaliar o potencial uso dos padrões de temperatura retículo-ruminal e atividade de novilhas Gir como preditores de parto e caracterizar a defesa e cuidado materno de vacas Gir, avaliando os possíveis efeitos da paridade e do protocolo de treinamento para a primeira ordenha nestes comportamentos. Foram utilizadas cinquenta e duas vacas Gir Leiteiro da Empresa de Pesquisa Agropecuária d Minas Gerais (Epamig Oeste, Uberaba, Brasil). Todas as amostras foram oriundas de um mesmo rebanho dividido em três grupos experimentais, um para cada capítulo. (Capítulo I): Quarenta novilhas Gir prenhes receberam um bolus intra-ruminal que registrou a temperatura retículo-rúmen (Trr) e atividade (Act). Os animais tiveram Trr e Act monitorados durante o período pré-parto. Observamos diminuição do Trr e aumento do Act nos dias que antecederam o parto. As diferenças em Trr e Act foram mais evidentes durante as últimas 21 e 11 horas antes do parto, respectivamente. Houve queda de 0,20°C na Trr. As análises revelaram que ambas as características têm potencial para predizer o parto, porém particularidades na fisiologia térmica de bovinos zebuínos devem ser consideradas quando se utilizam dispositivos validados apenas para raças europeias. (Capítulo II): Trinta e uma vacas Gir, dentre primíparas (n = 16) e multíparas (n = 15) foram alocadas em um piquete de maternidade monitorado por câmeras de vídeo. Os comportamentos dos animais foram coletados em quatro períodos: Pré-parto, Pós-parto, Primeiro manejo do bezerro e Pós-manejo. As vacas primíparas apresentaram maior duração dos comportamentos de ficar em pé com a coluna arqueada e tenderam a se movimentar mais do que as multíparas no período pré-parto, o que pode ser considerado indicador de dor e desconforto nesses animais. Tanto as primíparas quanto as multíparas foram protetoras de seus bezerros, mas apenas as multíparas foram agressivas com os tratadores no primeiro manejo do bezerro. Além disso, vacas mais protetoras passaram mais tempo comendo antes do parto, enquanto vacas menos atentas passaram mais tempo deitadas antes do parto. (Capítulo III): Trinta e sete vacas Gir leiteiras primíparas foram alocadas em dois grupos: O Grupo Treinamento (n = 16) foi submetido a um protocolo para a primeira ordenha, envolvendo estimulação tátil; O Grupo de controle (n = 21) foi submetido ao manejo comum da fazenda, sem interações e/ou manejos adicionais. Os comportamentos dos animais foram registrados em três períodos: pós-parto, primeiro manejo do bezerro e pós-manejo. A latência do bezerro para se levantar, o peso e o sexo influenciaram as interações vaca-bezerro. Vacas do Grupo Treinamento tocaram menos e passaram mais tempo sem interagir com seus bezerros. Ambos os grupos de treinamento e controle tinham mães protetoras, mas uma porcentagem maior de mães do Grupo Treinamento foram mais calmas em relação ao manejo dos bezerros. Em conclusão, Trr e Act apresentaram potencial para predição de parto em Novilhas Gir; Vacas Gir multíparas tendem a ser mais agressivas na proteção de seus bezerros do que primíparas; Protocolo de treinamento para a primeira ordenha reduziu o cuidado e defesa materna nas vacas Gir primíparas.

Palavras-chave: Agressividade. Amansamento. Reprodução. Zebu. Zootecnia de precisão.

ABSTRACT

The aim of this study was to evaluate the potential use of the reticulo-rumen temperature and activity pattern of Gyr heifers as calving predictors and characterize the defensiveness and maternal care of primiparous and multiparous Gyr cows, evaluating the possible effects of parity and training protocol to the first milking prior calving toward these behaviors. Fifty-two Gir Leiteiro cows from the Empresa de Pesauissa Agropecuária de Minas Gerais (Epamig Oeste, Uberaba, Brazil) were used. All samples came from the same herd divided into three experimental groups, one for each chapter. (Chapter I): Forty pregnant Gir heifers received an intra-ruminal bolus that recorded reticulo-rumen temperature (Trr) and activity (Act). The animals had Trr and Act monitored during the prepartum period. We observed a decrease in Trr and an increase in Act in the days before calving. Differences in Trr and Act were most evident during the last 21 and 11 hours before parturition, respectively. There was a drop of 0.20°C in Trr. The analyzes revealed that both characteristics have the potential to predict parturition, however particularities in the thermal physiology of Zebu cattle must be considered when using devices validated only for European breeds. (Chapter II): Thirty-one Gir cows, among primiparous (n = 16) and multiparous (n = 15) were allocated in a maternity paddock monitored by video cameras. The behavior of the animals was collected in four periods: Pre-calving, Post-calving, First handling of calf and Post-handling. Primiparous cows showed a longer duration of standing with an arched spine and tended to move more than multiparous cows in the pre-calving period, which can be considered an indicator of pain and discomfort in these animals. Both primiparous and multiparous cows were protective of their calves, but only multiparous females were aggressive towards the handlers in the first calf handling. Furthermore, more protective cows spent more time eating before calving, while less attentive cows spent more time lying down before calving. (Chapter III): Thirty-seven primiparous dairy Gyr cows were allocated into two groups: Training Group (n = 16) was submitted to a protocol for the first milking, involving tactile stimulation; Control group (n = 21) was submitted to the common management of the farm, without interactions and/or additional handling. Animal behavior was recorded in three periods: Post-calving, First handling of calf and Post-handling. Calf latency to stand up, weight, and sex influenced cow-calf interactions, whereas training group cows touched less and spent more time not interacting with their calves. Both Training and Control groups had protective dams, but a higher percentage of Trained group dams were calmer toward calf handling. In conclusion, Trr and Act had potential to calving prediction in Gyr Heifers; Multiparous Gyr cows tended be more aggressive with their calves' defense than primiparous; Training protocol to the first milking involving tactile stimulation reduced maternal care and defensiveness in primiparous Gyr cows.

Keywords: Aggressiveness. Livestock precision. Reproduction. Taming. Zebu.

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SUMMARY

1 GENERAL INTRODUCTION

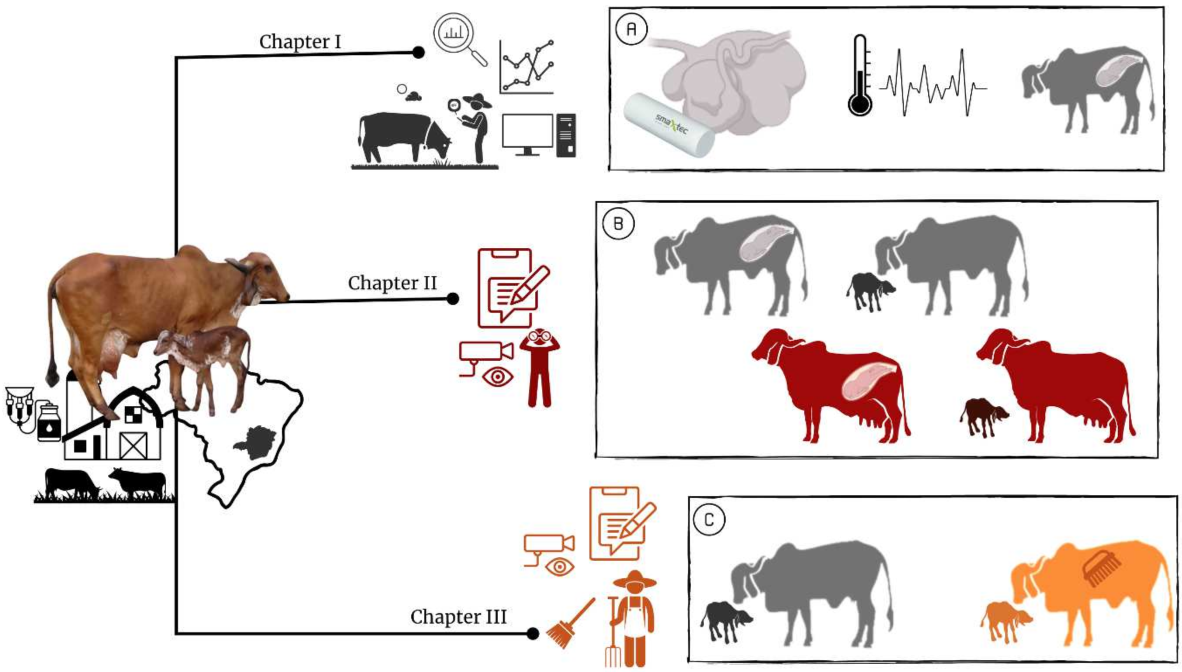
Trends such as public awareness and the advent of new technologies bring new demands for the Animal Production Industry. So, the farmers have also been adapting to this reality and acquiring a new profile, whether meeting a commercial demand, with the technification and automation of productive systems, or social, with the improvement of the human-animal relationships. These new approaches are favoring mainly large dairy properties that use taurine breeds (*Bos taurus taurus*), which are more technified and adopt intensive management. However, in developing countries like Brazil most rural properties are in the hands of small and medium producers that use Zebu-based breeds (1, 2).

In Brazil, livestock farming is one of the main economic pillars of the country. The agribusiness of milk and its derivatives plays an important role in the food supply and in the generation of employment and income for the population (3). The latest available data from the Food and Agriculture Organization of the United Nations (4) shows Brazil occupying the fourth position in the world milk production ranking. Brazilian dairy farming does not deviate from the demand for modernization observed in the world. Therefore, it is important to adapt and incorporate the new technologies and methodologies among tropical pasture-based systems. This modernization is not just an economic issue but also a social issue, leading small and medium-sized properties to new advances, culminating in also better economic development (2).

Like in other tropical countries, the Brazilian herd is composed mainly of Zebu cattle (*Bos taurus indicus*) raised extensively (5). Zebu animals are recognized by their adaptive attributes to tropical and subtropical environments because of their physiological and anatomical unique characteristics (6, 7). In addition, particularities regarding the behavior also are important in terms of production. Unlike taurine cattle, Zebu kind is described as more reactive and temperamental, frequently seen as more difficult to handle (8-10). While the Zebu's characteristics make it a good choice for tropical production, other biological and behavioral factors can be challenging in terms of applying new methodologies and techniques developed and validated for taurine cattle (6, 10, 11). In this context, studies that propose the modernization and improvement of production and handling processes involving Zebu cattle contribute to a more efficient and sustainable livestock in tropical dairy farming areas, mainly in developing countries.

This thesis presents three studies related to the use of precision technologies in calving prediction and the effects of parity and management on the behavior of Gyr cows in the peripartum period. The studies are organized in 3 Chapters. Chapter I describes the pattern of reticulo-rumen temperature and activity variation close to calving and the predictive potential of these traits for calving in Gyr heifers. Chapter II provides results of an investigation about the effects of parity on the behavior and maternal defensiveness of primiparous and multiparous Gyr cows during the peripartum period. Chapter III presents results of an investigation about the effects of a training protocol to the first milking involving tactile stimulation prior to calving on the behavior and maternal defensiveness of primiparous Gyr cows during the peripartum period. All samples were derived from the same herd divided into three experimental groups (Figure 1).

Figure 1 – Experimental design schematic representation used in reported studies which composes this thesis.



(A) Predictive potential of activity and reticulo-rumen temperature variation for calving in Gyr heifers (*Bos taurus indicus*); **(B)** Is maternal defensiveness of Gyr cows (*Bos taurus indicus*) related to parity and cows' behaviors during the peripartum period?; **(C)** Effect of training to first milking involving positive tactile stimulation on defense and maternal care at post-calving period in primiparous Dairy Gyr cows. Source: Personal Archive.

1.1 THEORICAL CONTEXTUALIZATION

Agriculture has modernized, but challenges remain. That is why there is a growing increase in research and studies that address both the use of new technologies and the improvement of living conditions in livestock. The following topics present a brief introduction of the main topics covered in this thesis.

1.1.1 GYR BREED

Brazil has the largest commercial herd in the world, with about 224.60 million heads (12). Among the breeds, Zebu cattle are expressive, considering the number of Zebu kind herds used in both beef and dairy farming (13). Zebu breeds represent those descended from South Asian cattle and are recognized by some particularities such as their long floppy ears and prominent hump (6). The main reason for the prevalence of Zebu cattle in the tropical dairy production is their rusticity, long-life spam, productivity, and fertility under unfavorable environmental conditions such as high temperatures, low-quality grasses, and parasites (14, 15).

Among all the Zebu breeds (e.g., *Brahman*, *Cagayan*, *Gyr*, *Guzerat*, *Indubrasil*, *Nelore*, *Punganur*, *Sindi*, *Tabapuan*), Gyr is the most used in crossbreeding and the most important in dairy farming, highlighted due to greater resistance and adaptability to adverse environmental conditions. Gyr breed is recognized by its good potential for milk production in extensive pasture systems, low maintenance requirement, high milk fat content, and resistance to ectoparasites and mastitis (16). This breed originated from Kathiawar peninsula (western India) and arrived in Brazil through importations that occurred between the 19th century, around 1870 and 1962, and since then have gained popularity and has been widespread across the whole country (17). With a wide variation in coat, the mocha variety (absence of horns) can also be found in the breed. According to the Associação Brasileria de Criadores de Gado Zebu (ABCZ), the breed had 807,790 animals registered in Brazil until 2020 [5]. The average production per lactation is 3,745.50 kg with 4.43% fat and 3.34% protein; average weight at weaning for males is 149 kg and for females 139 kg [5][14]. Figure 2 illustrates a typical Brazilian Gir cow (selected for milk production) and her calf.

Figure 2 – Cow and calf of Gyr breed (*Bos taurus indicus*).

Source: Personal Archive.

1.1.2 MANAGEMENT AND TECHNOLOGIES IN CALVING PREDICTION

The reproductive success of females is fundamental in dairy farming since milk production is dependent on conception, gestation, and parturition of cows. In cattle, gestation can range between 279 and 285 days, depending on maternal and fetal factors (18). Gestation culminates in the parturition itself, which is a sensitive period for cows. So, is important to monitor animal’s activity and behavior, especially close to calving, when animals are heavier and susceptible to obstetric issues. The attention at this moment should be higher for animals raised in grazing systems, where animals are exposed to weather and other environmental conditions.

In dairy production, cows spend the last weeks of gestation in separated paddocks, around 60 days before the calving prediction day for already milking cows (*i.e.* multiparous), and the whole gestation for heifers (*i.e.* nulliparous) (19). The farmers also translocate the animals to a maternity paddock close to the installations to facilitate the handling of calved cows and provide assistance in cases of calving problems. Usually, the identification of calving cows can be made by visual observation of the calving process or even the presence of the newborn itself (20). This process of identification can be labor-intensive, time-consuming, and lacks practicality in large dairy farms (21, 22).

Therefore, several technological approaches and tools have been developed for calving detection, driven by a movement called ‘livestock farming precision’ (23). These methodologies provide automated calving detection and/or prediction based on physiological and behavioral signs of the animals (11, 23, 24). So, these technologies are interesting tools that provide information in real-time, allowing more effective animal monitoring.

Among the physiological variables, body temperature is an easily measured parameter, being non-invasive, so it has been used in studies about the reproduction physiology (11, 25-28). Different body regions have been assessed in temperatures studies to predict calving, mainly focusing on taurine breeds: vaginal temperature (29, 30), ventral tail base surface temperature (31, 32), ear temperature (33), and reticulo-rumen temperature (25, 26, 34). In general, all studies described a decrease in body temperature ranging 0.2ºC to 1.0 ºC prior to calving, depending on the region and technology type used (25, 35, 36). This decrease is attributed mainly to the thermogenic effect of progesterone, which suddenly drops in the final stage of gestation and initiate the calving process (25, 35, 36). Among all available devices, reticulo-rumen temperature boluses are promising ones (37).

In addition, animal activity or movement can be remotely and automatically assessed using sensors that are also attached to devices internal to the animal’s body or to pedometers (38-41). In the hours prior to calving an increase in body movements and animal activity are observed, besides a decrease in water consumption and feed intake (42-44). The coupled accelerometers can identify the change in the pattern of animals’ activity, also using it as an indicator of calving process (41). Based on various analyses, some authors suggest that in calving prediction context, different types of measurements combined (*e.g.* body temperature plus animal activity) demonstrated better performance than each measure separately (11, 41). These new resources aim to assist in the management of herds, allowing accurate and reliable prediction in real time of crucial events that require management actions. In a practical context, a sensor that acts as a calving predictor could help to detect cows in prolonged and difficult calving processes, facilitating detection and management actions in cases of dystocia (26).

1.1.3 PERIPARTUM BEHAVIOR

At final gestation, maternal and fetal factors trigger endocrine events that start the calving process, being divided into three stages: 1) muscle contractions and dilation of the cervix; 2) fetus expulsion itself; 3) complete expulsion of placental remains (45-47). Each phase is characterized by specific behaviors of the cows, enabling the identification of the phase of calving.

The isolation behavior in the pre-calving period may be one of the first behavioral signs, indicating the beginning of calving process, and is influenced by several factors, such as paddock size, presence of shelter and vegetation (48-50). This is a strategy of wild ancestors that avoids predators' attacks on the offspring and may be preserved in domestic cattle (51). Although not all cows show this behavior, mainly in pasture-based farms it is a potential indicator used by handlers (48, 52). In the hours before calving, cows also become more restless and tend to decrease their water consumption and feed intake (42, 44, 53). As calving approaches and myometrial contractions intensify the animals change their activity patterns, in general increasing it (54). This increase in movements, stand and lying bouts and even vocalization during this period can be related to the pain and discomfort triggered by the contractions and cervix dilatation until the fetus expulsion (42, 55). Such behavioral aspects of calving process have been studied with the aim of characterizing and predicting the calving moment, besides verifying the influence of management, and predicting possible obstetric complications (52, 55-58).

After the fetus expulsion, the cow's attention tends to focus on the newborn, and at this moment began the formation of the mother-calf bond (59). This bond mother-son, also called imprinting, occurs through olfactory, visual, and auditory stimuli that result in reciprocal recognition (60). In this period both cow and calf have important roles to ensure reciprocal interest and consequently form a strong bond. This bond is extremely important to ensure better chances of calf survival and development (61). The first minutes after calving the cow stimulates the newborn with its tongue and muzzle, encouraging it to stand up (59). Additionally, the mother cleans the offspring of fetal membranes and eventually ingests it (54). In general, more experienced cows have less time to carry out the investigation and stimulation of the calf than primiparous and less experienced cows (62). In addition to parity, genetic and environmental factors can also influence maternal behavior (63). As soon as the calf stands up, it starts to search for the teats, nuzzling the cow's body (64). The first suckling latency is crucial for the survival of calves, as colostrum intake influences directly the immune acquisition (64, 65). Both latencies to stand up and first suckling reflect the calf vigor (66). Vigorous calves are those that have lower latency to stand up and lower latency to play the first suckling (67).

It is known that Zebu cattle, unlike taurine breeds, generally have particularities regarding behavior, with the expression and particularities of maternal-offspring behavior being one of the most influential in the productivity of the cows (68, 69). Unlike dairy farms that use European breeds and perform the separation of calves in the first 24 hours, for most Zebu dairy farms the cow-calf bond is extremely important (70, 71). On these farms, Zebu cows are usually milked with their calves and this kind of separation may compromise the milk ejection and the persistence of lactation (10, 72). Thus, for Zebu dairy cows the post-calving time is a sensible period and the handling of both cow and calf can be a challenging situation.

1.1.4 FIRST HANDLING OF CALF AND MATERNAL DEFENSE

The expression of maternal care is an essential component for the survival and development of the calf (59). Soon after calving, cow and calf maintain close spatial relationships and frequent communication after the postpartum period (61). Being prey species like other big herbivores, the calf is always under the constant care of the cow which provides protection against attacks by predators and/or conspecifics during the early stages of life (59). In cattle, mainly in Zebu cows, maternal care is so important that in cow-calf herds there is communal rearing groups in which the calves are under the protection of other cows while their mothers are grazing away (73).

Although the maternal defense importance to ensure greater chances of offspring survival, is possible that some cows show extreme protective responses, such as threatening behavior and/or attacking the handlers during the first handlings with the calves (49, 63, 74). Since in dairy Zebu herds, there is no separation of calves in the first hours of life, the first human-calf contact after calving can be a challenging handling situation for both handlers and cows. In practical terms, the newborns' handling is a necessary procedure and usually includes navel asepsis, weighing, and identification (75). However, some cows that perceive this type of management as a potential threat to the calf may exhibit extreme protective behaviors (74, 76). These reactions are undesirable because increase the risk of accidents with the handlers and animals (77, 78). Different characteristics of genetic and management origin can influence the cow's responses to the calf and the handling (59). The cow's response to their newborn and to the handling can be used to characterize their maternal style.

Previous studies investigated the maternal defensiveness behavior in cattle herds, mostly focusing on beef breeds, and only a few were conducted with dairy breeds (76, 79-81). For Zebu cattle, the breeds studied were beef Gyr, Brahman, and their crossbreed cows (74, 80) and Holstein-Gyr crossbred dairy cows (81). The results showed a positive relationship between cows reacting more protectively to separation from their calves and aggressive behaviors toward the handlers (80). Similarly, cows characterized as more 'frightened' and 'active' with their calves based on the qualitative assessment of behavior (QBA) that were also regarded as the most aggressive with handlers during the handling of calves (81). In general, Zebu cows are described as being more protective and in a dairy farming context, the impacts of this extreme protective behavior can be even worse as the animals are handled every day. These issues related to the maternal defensiveness and handling of calved cows are important in the livestock dairy industry, mainly to the Zebu-based breeds once aggressive cows can compromise the security and sustainability of the farms (63, 74, 77, 80).

1.1.5 HABITUATION AND TRAINING PROTOCOLS

Changes in the quality of the relationship between animals and handlers can influence the productivity and welfare of dairy farms (69, 82). Animals may perceive interaction with humans as negative, neutral or positive. According to Mota-Rojas et al. (83) the difference between them is mediated by the fear intensity as: moderate/high level with avoiding response (negative interaction); low level with avoiding response (neutral interaction); absence of fear with allowance of physical contact (positive interaction). In general, handling procedures that include shouts, kicks and/or tools that cause physical injury can be considered negatives with higher fear level (83, 84). On the other hand, the handling procedures that includes petting, calm voice, and other good practices can be considered positive with good welfare level (10, 83, 85).

Important changes in the sense of improving human-animal relationships can be obtained through habituation, training protocols, or other forms to reduce aversiveness (10, 86). Grissom et al. (87) defined habituation as decreased responsiveness to repeated stimuli. This type of non-associative learning process has been used to reduce the animals' fear in several situations, mainly those related to novelty and physical touch during handling (88). There are different habituation approaches also called 'positive tactile stimulation', 'positive handling', 'training', or 'training protocols' in different animal husbandry contexts (86, 88-91).

In dairy cattle, tactile stimulation and vocal interaction seem to be the most used and with better results (92). Most of these protocols are introduced gradually to animals. The benefits of this type of management can be more evident in dairy animals since they are handled every day at milking time and have direct contact with the handlers (10). In the scientific literature different methodologies are found in studies with cows of different parities in both European and Zebu breeds. In dairy Zebu cattle, the mostly breeds used are Gyr cows and Holstein-Gyr crossbreed cows (10, 88, 91). Previous results demonstrated a good performance of trained animals, mainly regarding the reduction of reactivity at milking time (10, 88). Although the adoption of these habituation protocols demonstrates promising results regarding the behavior and productive performance of the cows, the impact of this type of handling in other management contexts is not yet known. However, it may be possible that beside decrease the aversiveness and fear of human presence, the positive handling as habituation protocols would decrease the aggressive responses in calved cows (80).

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2 OBJECTIVES

2.1 MAIN OBJECTIVE

To evaluate the potential use of the reticulo-rumen temperature and activity pattern of Gyr heifers as calving predictors, as well as to characterize the defensiveness and maternal care of primiparous and multiparous Gyr cows, evaluating the possible effects of parity and training protocol to the first milking involving tactile stimulation prior calving toward these behaviors.

2.2 SPECIFIC OBJECTIVES

2.2.1 CHAPTER I

- To describe the pattern of reticulo-rumen temperature and activity variation in nulliparous Gyr heifers close to calving;
- To evaluate the predictive potential of these traits for calving in Gyr heifers.

2.2.1 CHAPTER II

- Investigate the effects of parity on the behaviors of Gyr cows during the peripartum period;
- Characterize the maternal defensiveness of primiparous and multiparous cows towards the handlers during the first handling of their calves;
- Evaluate the relationships between cows' behaviors at the peripartum period and maternal defensiveness.

2.2.1 CHAPTER III

- To investigate the effects of a training protocol to the first milking involving tactile stimulation prior to calving on maternal care of primiparous Gyr dairy cows during the post-calving period;

- To evaluate the effect of a training protocol to the first milking involving tactile stimulation prior calving on maternal defensiveness of primiparous Gyr dairy cows towards the handlers during the first handling of their calves.

3 CHAPTER I

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Predictive potential of activity and reticulo-rumen temperature variation for calving in Gyr heifers (*Bos taurus indicus*)

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ABSTRACT: Factors related to the thermal physiology and activity of Zebu animals close to calving are still unknown. The aims of this study were 1) to describe the pattern of reticulo-rumen temperature and activity variation in nulliparous Gyr heifers close to calving, and 2) to evaluate the predictive potential of these traits for calving in Gyr heifers. Forty pregnant Gyr heifers that had calved between August and December 2017 at the Getúlio Vargas Experimental Station, Empresa de Pesquisa Agropecuária de Minas Gerais (Epamig), Brazil, were used. The animals received a rumen bolus to monitor reticulo-rumen temperature (Trr) and activity (Act) at intervals of 10 minutes. Mixed linear models were used. A decrease in Trr and an increase in Act were observed on the days preceding calving. Differences in Trr and Act were more evident during the final 21 and 11 hours previous to calving compared to 14 days before calving, measured at the same time of day. There was a decrease of about 0.20ºC in Trr at the time of calving when compared to baseline (14 days before calving measured at the same time of day). Environmental variables, such as temperature and air humidity, as well as biological factors such as circadian rhythm, may influence the interpretation of the data. In conclusion, variations exist in the Trr and Act of Gyr heifers in the hours before calving, which is preceded by a decrease in Trr and an increase in Act. Particularities in the thermal physiology of Zebu cattle must be considered when prediction devices previously validated only for European breeds are used.

Keywords: dairy cattle farming; precision livestock farming; reproductive management; thermal physiology; Zebu cattle.

1 INTRODUCTION

The productive performance of cattle is directly related to reproductive success, which is determinant for the lactation of cows. Calving disorders can have genetic and non-genetic causes and can compromise the survival of the cow and calf (1). Monitoring animals during labor helps identify these possible problems and enables to provide obstetric assistance when necessary. However, the in-person inspection of the calving process is time consuming and labor intensive. Therefore, animal monitoring technologies such as temperature sensors and sensors recording behaviors such as feed intake, rumination and activity have been gaining space on farms, assisting with their productive management by predicting events such as estrus (2, 3) and calving (4-7) Precision techniques that assist in the automated detection of events related to animal reproduction, mainly the calving, contribute to herd management since they enable the accurate and reliable real-time prediction of key events that require management actions.

Changes in the movement and activity of the animal can help predict calving (5). Huzzey et al. (8) reported a tendency towards longer standing times of Holstein cows during the prepartum period. In the hours before calving, an increase in activity and movement is observed, as well as a decrease in water and feed intake (7, 9-11). In addition to behavioral parameters, body temperature is also a potential predictor of calving (12). Several studies have demonstrated decreases in the body temperature of cattle during the period preceding calving that ranged from 0.20 to 0.70ºC, measured at different sites. These decreases were found in crossbred beef (13) and Angus cows (2), as well as in dairy purebred Holstein (4, 14-17) and Japanese Black x Holstein cows (18). Regarding the sites and devices used for automated temperature monitoring in cattle, most of the studies have employed intravaginal temperature data (6, 15, 16, 18). However, intravaginal devices pose a risk of irritation and infection, a fact that renders them less attractive to producers and because of animal welfare issues (16). In view of the need for other noninvasive devices for automated temperature monitoring, reticulo-rumen devices have emerged as a viable alternative but are still insufficiently studied (12). The few studies using this technology suggest it to be promising for Holstein animals (2, 4, 17). However, it remains unknown whether these devices will also be useful for calving prediction in dairy Zebu cattle and under tropical farming conditions.

Zebu cattle (*Bos taurus indicus*) are widely used for livestock farming in tropical countries and the Gyr breed is an important dairy genetic resource (19). These animals possess characteristics that favor their adaptation to tropical and subtropical environments, especially features related to thermal physiology (19-21). Considering the physiological and behavioral particularities of Zebu cattle, studies on thermal physiology close to calving may contribute to the validation of technologies initially developed for European cattle (*Bos taurus taurus*) in Zebu animals. To investigate the predictive potential of the reticulo-ruminal temperature and activity devices as calving predictors in Zebu cattle, first, it is necessary to describe the pattern of reticulo-rumen temperature and activity variation along the late pregnancy period of the cows. Later, modeling should be used to test the quality of the best predictive models and algorithms for calving using the temperature and activity data of Zebu females.

Thus, the aims of the present study were 1) to describe the pattern of reticulo-rumen temperature and activity variation in nulliparous Gyr heifers close to calving, and 2) to evaluate the predictive potential of these traits for calving in Gyr heifers.

2 MATERIAL AND METHODS

The study was approved by the Ethics Committee of Animal Use of the Instituto de Zootecnia, Sertãozinho, São Paulo, Brazil (CEUA/IZ 230-16).

2.1 ANIMALS

Forty pregnant nulliparous Gyr heifers aged 30 to 90 months, which had their first calving between August and December 2017, were used. The experiment was conducted at the Getúlio Vargas Experimental Station, Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), Uberaba, Minas Gerais, Brazil (19º 44' 54" S latitude and 47º 55' 55" W longitude, altitude of 801 m).

Thirty days before the predicted calving date, the animals received an individual identification using non-toxic ink and were transferred to the maternity paddock where video cameras (GIGA, GSHDP20TB) were installed at strategic sites in order to record the exact moment of calving (date and time) of each animal. The maternity paddock was composed of *Urochloa decumbens* pasture and provided with natural shade (*Delonix sp.*). Nutritional management of the animals in the maternity consisted of corn silage and 500 g concentrate per animal, offered twice a day, in addition to water and mineral salt *ad libitum.*

2.2 METEOROLOGICAL VARIABLES

According to the Köppen classification, the climate of the region is of the subtropical *CWa type, with warm and rainy summers and relatively dry winters. During the experiment, minimum and maximum environmental temperatures and relative air humidity (RH), wind velocity, solar radiation and rainfall were collected at a meteorological station (A568) of the National Institute of Meteorology. The temperature-humidity index (THI) was calculated from the meteorological variables using the formula proposed by Thom (22): $THI = [0.8 \times T + (\%RH/100) \times (T-14.4) + 46.4]$, where T is the temperature (°C) and %RH is the relative humidity in percentage. Meteorological variables, including rainfall occurrence, varied across the experiment period. All meteorological variables collected are shown in Table 1.

Table 1 - Observed mean and standard deviation of ambient temperature, relative humidity, wind velocity, solar radiation and temperature humidity index over period of calving in Gyr heifers by week.

Week	Temperature (ºC)	Relative humidity (%)	Wind velocity (m/s)	Solar radiation (KJ/m²)	Rainfall (mm)	THI
1	18.46 ± 5.65	53.57 ± 18.54	2.59 ± 1.46	803.52 ± 1077.73	0.00	63.34
2	22.78 ± 5.30	47.81 ± 16.40	2.64 ± 1.51	764.85 ± 1116.81	0.00	68.62
3	22.06 ± 4.41	62.77 ± 19.59	2.54 ± 1.60	551.21 ± 869.89	0.00	68.85
4	22.22 ± 4.65	47.71 ± 15.45	3.47 ± 1.65	863.09 ± 1131.79	0.00	67.91
5	22.47 ± 5.42	37.66 ± 14.05	3.40 ± 1.48	908.43 ± 1210.62	0.00	68.58
6	22.84 ± 5.64	36.04 ± 12.62	3.26 ± 1.39	901.80 ± 1246.70	0.00	67.71
7	24.06 ± 5.64	36.24 ± 12.80	3.58 ± 1.31	938.94 ± 1268.39	0.00	69.14
8	24.28 ± 4.48	37.91 ± 12.02	3.65 ± 1.50	920.16 ± 1207.92	0.00	69.57
9	22.95 ± 4.83	57.80 ± 23.35	2.85 ± 1.36	734.93 ± 1090.71	0.04 ± 2.46	69.69
10	24.24 ± 5.35	56.84 ± 21.28	3.03 ± 1.83	1031.53 ± 1264.08	0.04 ± 0.22	71.38
11	27.53 ± 4.98	42.48 ± 15.44	2.83 ± 1.65	1353.71 ± 1706.77	0.03 ± 0.30	74.00
12	24.64 ± 4.47	56.53 ± 20.96	3.19 ± 1.83	1017.47 ± 1405.15	0.07 ± 0.76	71.89
13	24.68 ± 4.45	65.15 ± 20.12	2.57 ± 1.42	1105.58 ± 1596.45	0.27 ± 1.05	72.83
14	22.91 ± 3.22	75.31 ± 14.64	2.74 ± 1.62	1003.00 ± 1469.92	0.29 ± 1.29	71.13
15	23.47 ± 3.24	70.71 ± 17.94	2.94 ± 1.51	1007.68 ± 1424.65	0.06 ± 0.38	71.58
16	23.81 ± 3.95	67.81 ± 19.14	2.40 ± 1.66	960.79 ± 1245.76	0.12 ± 0.69	71.83
17	22.89 ± 3.18	81.23 ± 14.18	2.33 ± 1.33	829.03 ± 1269.83	1.71 ± 7.61	71.61
18	23.35 ± 3.76	81.39 ± 16.91	2.01 ± 1.10	892.12 ± 1196.50	1.10 ± 3.76	72.36
19	23.86 ± 2.84	81.20 ± 15.17	1.59 ± 1.05	757.06 ± 1045.24	0.18 ± 0.71	73.16
20	23.56 ± 3.42	73.96 ± 14.67	3.02 ± 1.37	1018.43 ± 1259.39	0.13 ± 0.86	72.01
21	23.54 ± 3.63	77.27 ± 15.48	1.91 ± 1.11	981.49 ± 1319.55	0.21 ± 0.79	72.29

THI: temperature humidity index. Source: Vicentini et al. (23)

2.3 DETERMINATION OF RETICULO-RUMEN TEMPERATURE AND ACTIVITY

Activity and temperature sensors in bolus form (TX-1442, Smaxtec Animal Care, Austria) were administered into the reticulo-rumen region of heifers in September 2016. The implantation of bolus was performed by oral route using a custom balling gun, following manufacturer recommendation, while the heifer was restrained at squeeze chute. The bolus device had a dimension of 105 mm x 35 mm (length x diameter), 0.21 Kg and was ruminal fluid resistant. After administration of the bolus, the animals were allowed to adapt for 45 days and were then submitted to fixed-time artificial insemination (FTAI). Pregnancy was diagnosed 30 days after FTAI by transrectal ultrasound (Siui CTS 900v). Animals that did not conceive were inseminated two more times (with the diagnosis of pregnancy 30 days after each insemination) and then transferred to natural mounting until pregnancy was confirmed by ultrasound.

The measurement scale of the temperature sensors ranged from 0°C to 50°C (± 0.25°C). Animal activity was measured with accelerometers (inside the bolus) that generated an activity index (from 0 to 100%). The reticulo-rumen temperature (Trr) and activity (Act) were measured at intervals of 10 minutes. The data were collected with a telemetry system during pregnancy. The range of operation of the antenna was 30 m. The antenna was placed in the pen while the animals were managed. The readings obtained with the antenna were sent to online servers (with cloud storage of the data) via a radio signal and were accessed by the online Smaxtec Messenger software (Smaxtec Messenger, Smaxtec Animal Care, Austria).

2.3 STATISTICAL ANALYSIS

The Trr and Act data were recorded every 10 min. by the bolus devices. All datasets were submitted to descriptive analysis and edited for statistical analysis by calculating the temperature and activity per hour (averages of 10 min. values collected). Values of Trr less than 37.72°C were discarded in order to minimize the influence of water intake (4, 24).

The mean values of Trr and Act per hour over the six days preceding calving (6 measurements per hour) were calculated for statistical analysis in order to compare these parameters at different moments before calving. The data were edited using the R program (R Core Team, 2019). First, analysis of variance of each variable was performed, considering Trr and Act as dependent variables. To evaluate each variable, the days before calving (-6, -5, -4, -3, -2 and -1 days and day 0 defined as day of calving) were included as fixed effects, and THI and time of day as covariates with linear effects. The heifer's age was previously included as a covariate, however, it was not significant ($P > 0.05$) and thus it was removed from final analysis.

Next, the Trr and Act data were divided into two separate datasets: 'CALVING', including data from the 48 consecutive hours preceding the day of calving; 'NON-CALVING', including data from the 48 consecutive hours preceding day 14 prior to calving, which is an adaptation of the "same hours method" proposed by Aoki et al. (18). The method is based on a comparison between the Trr and Act at a particular time of the day previously to birth (CALVING condition) with the same time of the day on the 14º day preceding to calving (NON-CALVING condition) to ensure that the cow was not in labor. Mixed linear models were applied separately to the two datasets ('CALVING' and 'NON-CALVING') to analyze the variation in Trr and Act over the hours before calving using the PROC MIXED procedure of SAS (SAS® Institute, Inc., Cary, NC). The dependent variables were Trr and Act. The 'hour before calving' was included as fixed effect in the model and 'time of day' and 'THI' as linear covariates. The 'animal' was included as random effect repeated on 'hour before calving'. The repeated measure of the animal at the different time points was modeled using different residual (co)variance structures (ANTE, AR (1), VC, CS, CSH, and TOEPH) and the best structure was chosen based on Schwarz's Bayesian information criterion as described by Wolfinger (25). According to this criterion, an ante-dependence (ANTE) variance structure was chosen for Trr and a heterogeneous Toeplitz (TOEPH) structure for Act.

The random forest method was applied to verify the possibility of calving prediction using the 'randomForest' package of the R program (R Core Team, 2019). The response variables were assumed to have a binary distribution, in which $Y_i = 1$ was defined as the calving hour (immediately before the event) and $Y_i = 0$ as the other hours. The 24 and 36 hours before calving were considered for analysis. The signaled response variable for prediction ($Y_i = 1$) were the periods (2, 4, 6, 8, 10, and 12 hours) preceding calving. Three models were considered for analysis: Model 1 containing Trr and Act data; Model 2 containing only Trr data, and Model 3 containing only Act data.

The three models also included the effects of ‘time of day’ and ‘THI’ as independent variables.

The predictive potential of the models was calculated using training and validation datasets as described by Borchers et al. (5). For this purpose, the datasets were randomly divided into five groups of eight animals each, following the recommendations by Borchers et al. (5). The effects of the predictors were estimated in four groups (80% of observations), defined as the training set, and the predictive ability of each model was tested in one group (20% of observations), called test subset. The analyses were repeated five times; in each repetition, a different group was considered the test population and the remaining groups the training population. After the prediction for each test subset, assertive responses were generated using true positives (TP), false positives (FP), true negatives (TN), and false negatives (FN). The following metrics were calculated to evaluate the prediction performance of the different models according to the method of Borchers et al. (5): sensitivity: TP/(TP + FN) × 100; specificity: TN/(TN + FP) × 100; positive predictive value (PPV): TP/(TP + FP) × 100; negative predictive value (NPV): TN/(TN + FN) × 100. As auxiliary measures, the area under the ROC curve (AUC) (ROCR package of the R program) and the model accuracy [TP + TN/(TP + TN + FP + FN)] were calculated. The mean result of the five test populations was used as the final performance metric. The AUC results were classified according to Zhu et al. (26): excellent: 0.9 < AUC < 1.0; good: 0.8 < AUC < 0.9; worthless: 0.7 < AUC < 0.8; not good: 0.6 < AUC < 0.7.

3 RESULTS

First, the variation in daily Trr and Act was evaluated over the six days prior to calving. The mean Trr and Act of the six days before calving were 39.28 ± 0.05ºC and 8.15 ± 0.29%, respectively. Analysis of variance showed a significant effect of days before calving (F = 4.75; P < 0.01), time of day (F = 1320.09; P < 0.01), and THI (F = 258.35; p < 0.01) on Trr. Similarly, daily Act was also influenced by days before calving (F = 12.05; P < 0.01), THI (F = 315.9; P < 0.01), and time of day (F = 86.86; P < 0.01). There was a decrease in Trr as the day of calving approached, while the opposite was observed for Act, with higher values closer to the day of calving (Table 2).

Table 2 - Estimated daily means of reticulum-rumen temperature (Trr) and activity (Act) on the six days before calving in Gyr heifers.

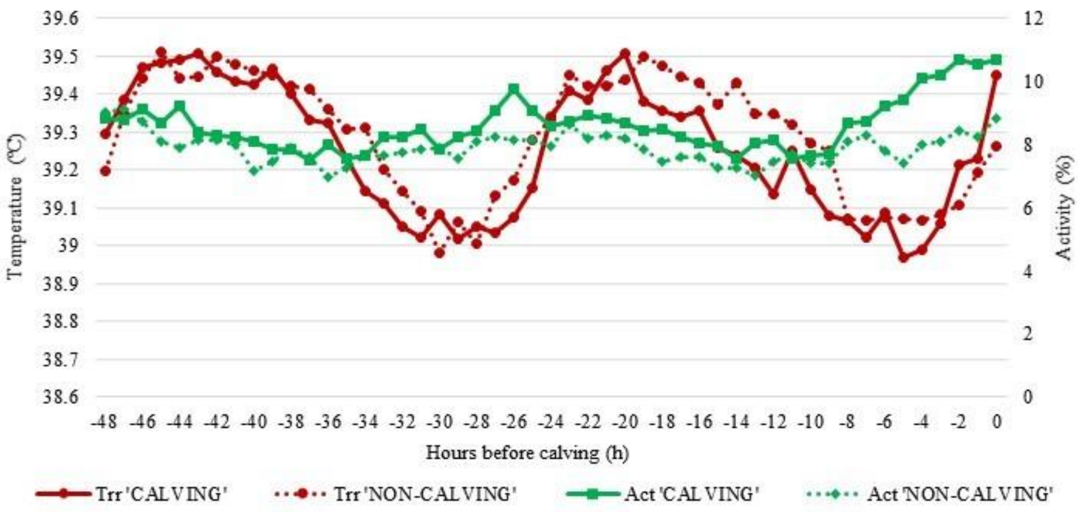
Day before calving	Trr (ºC)	Act (%)
0	39.23±0.01[c]	8.74±0.08[a]
-1	39.27±0.01[bc]	8.33±0.08[b]
-2	39.27±0.01[abc]	8.09±0.08[bc]
-3	39.30±0.01[ab]	8.14±0.08[bc]
-4	39.33±0.01[a]	7.97±0.08[bc]
-5	39.32±0.01[ab]	7.94±0.08[c]
-6	39.30±0.01[ab]	7.90±0.08[c]

[a – c] Different superscript letters in the same column indicate significant differences by the Tukey test ($P < 0.05$). Source: Vicentini et al. (23)

The analyses showed a significant difference in mean Act on the day preceding calving compared to the other days (Table 2). For Trr, the estimated means were statistically similar on the two days preceding calving and on the day of calving.

To better illustrate the Trr and Act variations over time, the means per hour were estimated over the 48 hours preceding calving ('CALVING' period) and the same 48 hours 14 days prior to calving ('NON-CALVING' period). The results are shown in Figure 1.

Figure 1 – Estimated means per hour of reticulo-rumen temperature (Trr) and activity (Act) over the 48 hours preceding calving ('CALVING') and over the 48 hours 14 days prior to calving ('NON-CALVING') in Gyr heifers.

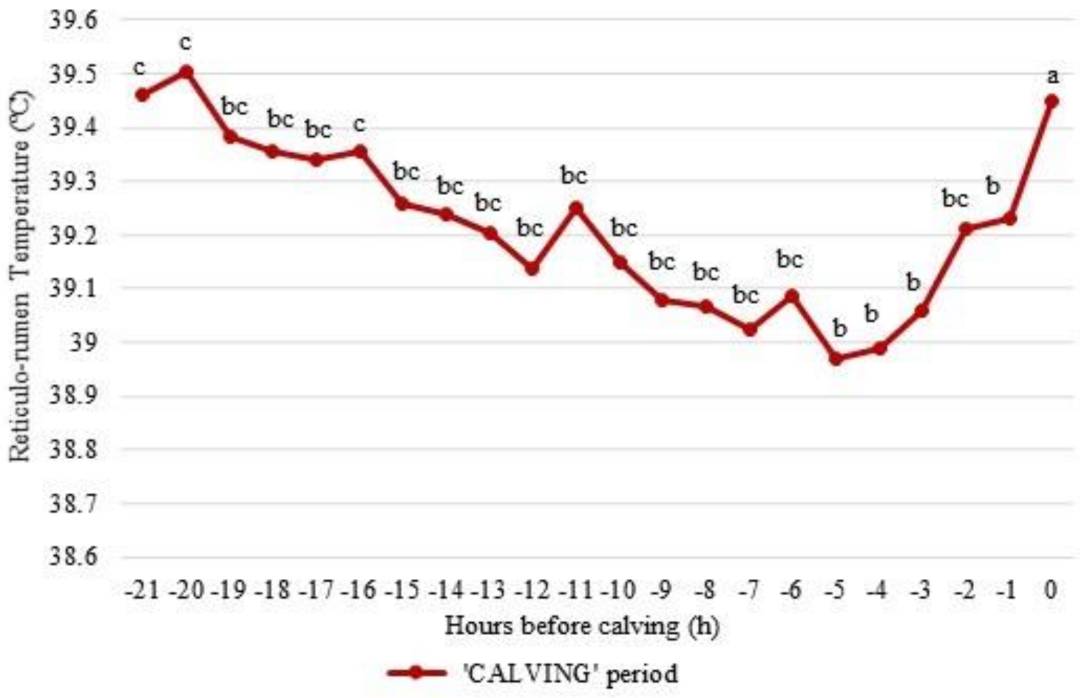


Source: Vicentini et al. (23)

The variation in the two traits (Trr and Act) exhibited an inverse pattern, with a reduction in temperature and an increase in activity close to calving, demonstrating a double-sigmoid curve (Figure 1). For Trr, similar means were observed in the two periods; however, there were sudden temperature drops in the 21 hours preceding calving when compared to the same period 14 days before calving (‘NON-CALVING’). Analysis showed significant effects of hour before calving and THI (P < 0.01) for the ‘CALVING’ period, while only the effect of THI was significant (P < 0.01) for the ‘NON-CALVING’ period.

For the ‘CALVING’ period, the Tukey test revealed significant differences between the estimated means of Trr at the time of calving (time 0) and hours -1 (P = 0.02), -3 (P = 0.02), -4 (P = 0.01) and -5 (P = 0.01), with lower means for the latter hours (Figure 2). There were also differences in means between hours -4 and -20 (P = 0.03), -5 and -16 (P = 0.02) (Figure 2).

Figure 2 – Estimated means of reticulo-rumen temperature in the 21 hours before calving for the ‘CALVING’ period in Gyr heifers.

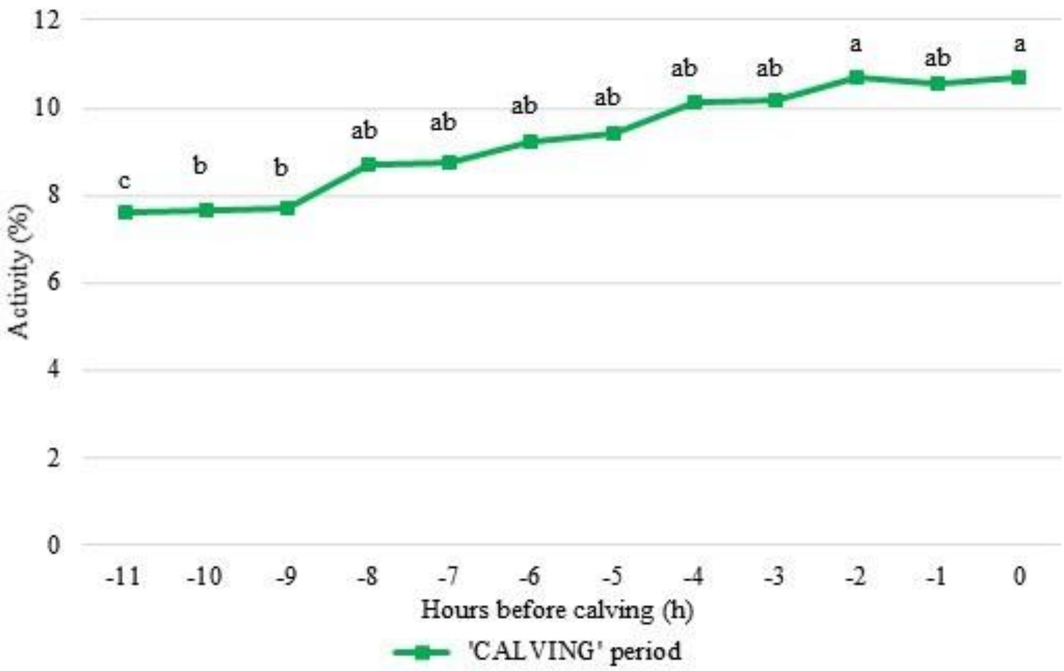


Source: Vicentini et al. (23)

For Act, there was a significant effect (P < 0.01) of hour before calving for the ‘CALVING’ period and only of THI for the ‘NON-CALVING’ period. In addition, an increase in Act was observed in the 11 hours preceding calving (Figure 3). Similar to

Trr, the Tukey test revealed significant differences in Act between the hours preceding calving: 0 and -9 (P = 0.03); 0 and -10 (P = 0.03); 0 and -11 (P = 0.01); -1 and -11 (P = 0.04); -2 and -9 (P = 0.03); -2 and -10 (P = 0.04), and -2 and -11 (P = 0.01) (Figure 3).

Figure 3 – Estimated means of activity in the 11 hours before calving for the 'CALVING' period in Gyr heifers.



Source: Vicentini et al. (23)

Since the analyses demonstrated changes in Trr and Act in the hours close to calving, the prediction models were tested considering data of Trr and Act (Model 1), only Trr (Model 2), and only Act (Model 3). Intervals of two hours up to the 12 hours preceding calving were defined as success for analysis (Yi = 1) in 24- and 36-hour sampling periods (Yi = 0).

For all models, the best metric results were obtained at 12 hours considering the 24-hour period (Table 3). Higher specificity and accuracy were found in the hours closer to calving, while the inverse phenomenon was observed for sensitivity for the three models tested. During this period, the AUC ranged from 0.52 to 0.71. The highest PPV was 67% obtained with Model 1 at 12 hours and the highest NPV was 88.33% obtained with Model 3 at 2 hours.

Table 3 - Prediction of calving using Trr and Act data for intervals of two hours up to the 12 hours before calving in Gyr heifers.

Signaled hours (Yi = 1)	Sampling period (Yi = 0)	Accuracy (%)	AUC	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
		Model 1 (Trr + Act)					
2	24 hours	87	0.69	5.4	98.5	34.0	88.1
4		80	0.71	13.6	96.4	46.4	81.3
6		74	0.71	30.6	91.6	61.0	76.9
8		69	0.69	43.3	84.1	61.9	72.2
10		66	0.68	57.9	72.1	62.8	68.1
12		67	0.70	72.1	60.6	67.0	66.3
2	36 hours	91	0.69	4.7	98.8	48.2	91.9
4		86	0.69	6.9	98.1	28.2	86.8
6		80	0.68	11.3	96.5	41.5	82.1
8		74	0.64	12.4	93.7	41.2	76.7
10		66	0.68	57.0	72.4	62.9	67.7
12		63	0.61	20.7	85.5	43.7	66.4
		Model 2 (Trr)					
2	24 hours	87	0.52	2.9	98.7	15.0	87.9
4		79	0.57	9.8	96.5	34.8	80.8
6		71	0.62	22.1	90.9	49.7	74.8
8		66	0.63	40.5	81.5	56.5	70.6
10		61	0.62	53.0	68.8	57.8	64.7
12		60	0.65	63.9	56.1	61.4	58.8
2	36 hours	87	0.50	2.9	99.1	20.0	87.9
4		78	0.58	8.9	96.4	32.0	80.6
6		72	0.62	23.2	91.0	51.4	75.1
8		66	0.63	39.7	81.2	56.0	70.1
10		62	0.63	53.0	69.2	57.9	65.0
12		59	0.64	63.6	55.9	61.2	58.4
		Model 3 (Act)					
2	24 hours	87	0.68	6.9	98.2	44.1	88.3
4		80	0.63	16.7	96.3	49.0	81.9
6		73	0.68	29.1	90.7	53.9	76.5
8		64	0.65	34.1	80.6	49.4	68.3
10		60	0.64	49.8	69.1	55.9	63.6
12		61	0.65	65.2	57.2	62.5	60.3
2	36 hours	87	0.68	7.6	98.3	46.0	88.4
4		80	0.68	18.0	96.4	50.1	82.1
6		73	0.67	28.8	91.2	55.3	76.5
8		63	0.65	32.3	81.3	48.9	67.9
10		60	0.64	50.1	69.2	56.0	63.9
12		61	0.65	66.7	55.5	62.0	60.8

AUC: area under ROC curve; Trr: reticulo-rumen temperature; Act: activity. Source: Vicentini et al. (23)

4 DISCUSSION

This study showed a consistent decrease in Trr and increase in Act as calving approached. These variations were more evident on the days preceding calving. Previous studies on taurine cattle (*Bos taurus taurus*) found a reduction in the body temperature of beef (2, 13) and dairy cows (4, 14). Variations in Act and Trr might be related to alterations in the metabolic profile and endocrine variations during the period preceding calving (2, 13).

The drop in Trr was more evident on the day before and on the day of calving, when the lowest mean temperatures were recorded (Table 2). There was a decrease of up to 0.20ºC at 14 hours before calving, a value lower than that reported in other studies on taurine cows (Figure 1). In Holstein cows, Burfeind et al. (15) observed a decrease of up to 0.7ºC in vaginal temperature and of up to 0.6ºC in rectal temperature 48 hours before calving. Similarly, Koyama et al. (27) reported a decrease of 0.6 to 0.9ºC in ventral tail base surface temperature of Holstein cows. Lower temperature drops were reported in studies using ruminal devices. For example, Cooper-Prado et al. (2) used temperature-sensing boluses placed in the rumen of Angus cows and observed a decrease of up to 0.33ºC two days before calving. Similarly, Costa Jr. et al. (4), who also used temperature-sensing boluses placed in the rumen of Holstein cows, reported Trr drops of 0.32ºC and 0.36ºC in primiparous and multiparous cows, respectively, 24 hours before calving. For crossbred Holstein-Japanese Black cows, Kovács et al. (17) found different drops in Trr depending on the calving condition, which ranged from 0.23ºC for distocic cows to 0.48ºC for eutocic cows. Thus, divergences in the literature data can be attributed to differences in the methods applied, such as the site of body temperature measurement, environmental variables and biological variables of the animal, factors that can influence body temperature (28, 29). Nevertheless, Trr remains proportional to the animal's core temperature and intraruminal devices are therefore considered appropriate to estimate variations in body temperature (30).

The significant effect of THI was attributed to the influence of temperature and humidity on body temperature (31). A previous study on Gyr cattle showed that the pattern of Trr varies according to season of the year, with pregnant Gyr cows exhibiting a higher mean ruminal temperature in winter compared to summer (32). The period and time of day can also influence body temperature measurements since these components of thermal physiology are coordinated by the circadian rhythm, with the observation of lower values in the morning and higher values in late afternoon (33).

The significant effect of days before calving on activity indicates greater movement of the animals on the days close to calving (Table 2), possibly as a result of the uterine contractions characteristic of labor (9, 34). Studies have reported changes in the behavior of cows on the days preceding calving, which are related to lower feed intake and shorter rumination time (5, 35, 36), increased activity, and alterations in the number of lying bouts (10, 37, 38). For dairy Holstein cows, the mean activity level of the animals recorded with neck-collar accelerometers was 2.9 ± 0.04 in the week before calving and increased to 9.1 ± 0.81 in the 5 hours preceding calving (39). Similar to the observation for Trr, the significant effect ($p < 0.01$) of time of day on Act can be attributed to the circadian rhythm of activity, especially that related to grazing and resting behaviors (40, 41). This variable was therefore included in the models as a covariate for the purpose of control.

Analysis of hourly variations over the 48 hours preceding calving (Figure 1) demonstrated the circadian rhythms of Trr and Act. For Trr, the same range of variation (38.97ºC to 39.51ºC) was observed in the two datasets ('CALVING' and 'NON-CALVING'). However, lower values and a more sudden drop 21 hours before calving were found for the 'CALVING' period. The literature reports drop in body temperature of up to 1ºC in the hours preceding calving, which are largely attributed to the thermogenic effect of progesterone (2, 14, 18). Hourly comparisons showed that Trr 5 hours before calving differed from Trr at the time of calving, when the highest temperature was recorded (Figure 2). The highest temperature at the time of calving may be explained by the higher activity level of the animal, which could be the result of the constant movement and changes in blood circulation caused by labor contractions of the uterus (9, 34, 42).

As demonstrated for Trr, variations in Act were also observed in the last 48 hours before calving (Figure 1). Cows tend to move more on the day before calving and spend more time standing (8). Comparison of the 'CALVING' and 'NON-CALVING' periods generally showed higher Act values for the former. There was an intense increase of Act in the last 11 hours before calving, which can be attributed to the greater movement resulting from labor, in agreement with the literature. Borchers et al. (5) observed a larger number of steps and general movement of Holstein cows in the hours before calving. Miedema et al. (9) also found a higher activity level hours before calving in cows of the same breed. Similarly, Jensen (10) reported a higher activity of Holstein cows 6 hours before calving compared to the same time three days prior to calving. In the present study, mean activities indicated a higher rate of movement in the last 8 hours before calving (Figure 3). This finding can be explained by the restless behavior of the animals when in labor, which exhibited characteristic signs of pain and discomfort, often observed through lying and standing behavior (8).

The prediction models of the performance metrics analyzed individually (Trr and Act) revealed satisfactory results. However, they should be considered in conjunction with one another, as combination of Trr and Act showed better predictions. In the present study, sensitivity is the ability of the model to correctly predict calving (Yi = 1) and specificity is the ability of the model to predict the non-occurrence of calving (Yi = 0); hence, high values were expected for both parameters. However, we observed an inverse variation of these parameters, *i.e*. models with higher sensitivity showed low specificity (Table 3). A similar phenomenon was found for PPV and NPV. The models with higher accuracy exhibited lower sensitivity, indicating that the latter may not be the best parameter for model evaluation. Thus, the model exhibiting the highest values of all metrics together (i.e., all metrics combined: accuracy, area under the ROC curve, sensitivity, specificity, positive predictive value, and negative predictive value) was defined as the most adequate.

When the calving prediction potential was evaluated, Model 2 and Model 3 showed a similar performance. However, Model 1, which combined the Trr and Act data, provided the best performance values (Table 3). Ouellet et al. (2016), using temperature, rumination, and movement sensors, also evaluated a combination of measures (rumination time, vaginal temperature, and lying behavior) for calving prediction in Holstein cows and obtained 77% sensitivity and 77% specificity. Borchers et al. (5) also reported satisfactory results combining measures collected by collars and accelerometers for monitoring activity and rumination (neck and rumination activity, number of steps, total movement, lying time, and lying bouts). The authors reported 82.8% sensitivity and 80.4% specificity for calving prediction in Holstein cows.

Regarding the area under the ROC curve, when the results were analyzed individually, performance was mostly classified as ‘not good’ (0.6 to 0.7) or ‘worthless’ (0.71 to 0.8) only for the 4, 6 and 12 hours evaluated with Model 1 (Yi = 24 hours). Costa Jr. et al. (4), also studying Holstein cows, reported AUC values of up to 0.75 using only Trr data. On the other hand, in the study of Burfeind et al. (15) evaluating equations to predict calving in Holstein cows using rectal and vaginal temperature, the AUC ranged from 0.77 to 0.87.

The divergence between the present results and those reported in the cited studies might be attributed to differences in the type of collected data, including temperatures measured at different sites (Trr, vaginal temperature, rectal temperature) and different types of animal activity (rumination time, number of steps, lying time, lying bouts), and their respective analysis methods (4, 5, 29). In addition, within each approach, the establishment of a threshold for changes in temperature and activity patterns depends on the number of observations, experimental conditions, environmental factors, type of management, and herd. Therefore, since we were able to identify minor variations in the internal temperature of Gyr heifers compared to that reported for taurine animals, the performance of algorithms developed for European cattle may be compromised when used by Zebu cattle.

In general, the patterns of Trr and Act variation of Gyr heifers close to the first calving were similar to those reported in the literature. The results showed that the activity of Gyr heifers close to calving was consistent with the findings for taurine animals. However, we observed a lower magnitude of Trr variation when compared to European cattle. This result might be explained by physiological differences among subspecies, especially those related to thermal physiology (21). These differences must be taken into account when technologies for calving prediction are used. Although our prediction equations are not suitable for use as a calving prediction algorithm in the field yet, the results provide information about Trr and Act variations that could be an indicator of calving in nulliparous Zebu heifers. In the future, farmers would benefit from intraruminal devices to predict the calving based on temperature, enabling earlier and more efficient calving assistance in Zebu cattle, as already available for European dairy breeds. However, for the application of this knowledge in the field focused on Zebu cattle, further studies on the components of thermal physiology close to calving are necessary to better understand the dynamics of variation at this moment and enable the development of an adequate predictive mechanism and algorithms.

5 CONCLUSION

In conclusion, changes occur in the Trr and Act of Gyr heifers in the hours before their first calving, which is preceded by a decrease in Trr and an increase in Act. The magnitude of the Trr drop in Gyr animals was lower than that reported for European cattle. Thus, physiological differences in thermal physiology between Zebu and European cattle should be taken into consideration when Trr is used for calving prediction.

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4 CHAPTER II

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Is maternal defensiveness of Gyr cows (*Bos taurus indicus*) related to parity and cows' behaviors during the peripartum period?

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ABSTRACT: The maternal care of cows can influence both the milk production and the performance of their calves, making this a topic of important relevance for the production industry that uses zebu cattle. The aims of this study were to 1) investigate the effects of parity on the behaviors of Gyr cows during the peripartum period; 2) characterize the maternal defensiveness of primiparous and multiparous cows towards handlers during the first handling of their calves; and 3) evaluate the relationships between cows' behaviors at the peripartum period and maternal defensiveness. Thirty-one Gyr cows (primiparous and multiparous), from Empresa de Pesquisa Agropecuária de Minas Gerais (Brazil), were used. The animals were placed in a maternity paddock monitored by video cameras. The behaviors of the animals were collected in four periods: Pre-calving, Post-calving, First handling of calf and Post-handling. Primiparous cows presented more pain signs, reflected in arched spine (P = 0.05), and tended to move more (P = 0.07) than the multiparous in the Pre-calving period. Trends were observed for both Maternal Composite Score (P = 0.06) and Maternal Protective Behavior score (P = 0.06), indicating that both primiparous and multiparous were protective, but only multiparous cows were aggressive toward the caretakers on the first handling of their calves. The most protective cows spent more time eating during the prepartum period (P = 0.03), while the least attentive cows spent more time lying down (P = 0.02) in the prepartum period. The cows who nursed and stimulated their calves more were also calmer (P = 0.02) and more attentive (P = 0.01). In conclusion, the peripartum behaviors of Gyr cows were related to maternal care and maternal defensiveness. Multiparous cows tended to be more aggressive than primiparous cows at the time of the first handling of their calves.

Keywords: Aggressiveness; Calf protection; Maternal care; Maternal protection; Zebu cattle.

1 INTRODUCTION

Despite the process of domestication and intense artificial selection in dairy cattle, resulting in several breeds and behavioral changes in relation to their wild ancestors (1), the behaviors related to maternal care and protection of offspring have been maintained in some breeds (2). These behaviors are important components and desirable for offspring survivorship and development in extensive cattle production systems (3-5). After birth, a strong mother-offspring bond is formed (6). The bond formation is mediated mainly by olfactory, visual, and auditory stimuli that result in reciprocal individual recognition (7). Good quality cow-calf interactions soon after birth are important to assure better chances of offspring survival (5). Experienced cows (*i.e.* multiparous) usually have a shorter latency to investigate and stimulate the calf compared to inexperienced cows (*i.e.* primiparous) (6, 8). In addition to the parity, genetic and environmental factors might also affect the maternal behavior of cows (9, 10). These behaviors, such as investigation, stimulation, maternal care, and defensiveness, can be used to characterize the maternal style, suggesting that these animals might have stable inter-individual differences that could be regarded as the maternal temperament (5, 11).

As a prey species, an important component of the maternal style is defensiveness since the calf should be under the dam's constant care in order to provide protection against predators and/or threatening conspecifics during the first weeks of life (12). Although maternal defense behaviors are necessary and desirable under more natural conditions, in farming production systems, cows displaying extreme protection responses in relation to their calf might threaten or attack handlers during the first handling of their calves. Such extreme reactions can raise the risks of on-farm accidents, injury to handlers and animals, and even threaten the calves' welfare, leading to physical damages or abandonment of calves (4, 13).

Previous studies have aimed to investigate the maternal care and the expression of maternal protective behaviors in cattle herds, mainly focusing on beef cattle breeds (3, 4, 9, 14-17), and only a few have been conducted with dairy cattle (18, 19). This is a relevant question in cow-calf contact dairy systems. For Zebu cattle (*Bos taurus indicus*), the maternal defensiveness behavior was assessed in beef Gyr, Brahman, and their crossbreed cows (14, 17) and Holstein-Gyr crossbred dairy cows (19). In the study of Pérez-Torres et al. (14), 90% of the cows displayed defensive behavior when the handler was close to the calf at 30 days after birth. These defensive reactions were strengthened when the calves vocalized or were handled. It is possible that the cows perceived humans as potential predators (14). Evaluating multiparous cows, Orihuela et al. (17) reported that cows reacting more protectively to separation from their calves also exhibited more aggressive behaviors towards the handlers. However, the author failed to find a relationship between maternal protectiveness and the cows' temperament in the peripartum period (17). Ceballos et al. (19) reported that Holstein-Gyr crossbred cows who were more aggressive during the handling of their calves tended to be characterized as more 'frightened' and 'active' than those regarded as 'loving' and 'attentive' towards their calves when assessed using a qualitative behavior assessment, evincing a possible relationship between maternal defensiveness and the maternal style of care.

These issues are relevant in dairy herds of Zebu dairy herds in which the calves are not separated from their dams early post-birth, as is typical in European dairy breeds (*Bos taurus taurus*). The lack of stimuli from the calves compromises the length of the lactation period, that can be shortened, in Zebu cows, therefore the use of cow-calf contact systems is common for these animals (10, 20). In dairy systems using Zebu breeds, the maternal defense and the mother-offspring bond are important, since the cows are known to not fully adapt to machine milking and being milked with their calves (21, 22).

Thus, the aims of the present study were to: 1) investigate the effects of parity on the behaviors of Gyr cows during the peripartum period; 2) characterize the maternal defensiveness of primiparous and multiparous cows towards the handlers during the first handling of their calves; and 3) evaluate the relationships between cows' behaviors at the peripartum period and maternal defensiveness.

2 MATERIAL AND METHODS

The experiment was conducted at the Getúlio Vargas Experimental Station, Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), Uberaba, Minas Gerais State, Brazil (19º 44' 54" S latitude and 47º 55' 55" W longitude, altitude of 801 m) and was approved by the Ethics Committee of Animal Use of the Instituto de Zootecnia, Nova Odessa, São Paulo State, Brazil (CEUA/IZ 230-16).

2.1 ANIMALS AND HANDLING

Thirty-one Gyr cows (*Bos taurus indicus*), primiparous (n = 16) and multiparous (n = 15) were used. The animals were aged between 30 to 132 months and calved between July and December 2017. The calving order of multiparous cows ranged from two to six calvings. Thirty days before the estimated calving day, cows were transferred from the pasture to a maternity paddock of 0.55 ha size. The maximum stocking density in the maternity paddock was 27.27 animals/ha, and all cows had access to natural shade. The paddock was covered with *Urochloa decumbens* grass. Cows were fed with corn silage and 500 g of concentrate/head delivered twice a day, in addition to mineral supplements and water *ad libitum*. During the study period, cows were not handled or disturbed. Only routine procedures (feeding, calves' identification, and navel disinfection) were conducted by the usual familiar handlers.

2.2 BEHAVIORAL OBSERVATIONS

In the maternity paddock, cows were individually identified with non-toxic paint. Four monitoring cameras (GIGA, GSHDP20TB) were installed in the paddock corners to record the cows' behaviors 24 hours a day.

In this study, only eutocic and non-twin calvings were included. The calving moment was defined as the complete expulsion of the fetus. After calving, a minimum period of 3 hours was permitted for cows and calves to remain together without any human disturbance. Afterward, the first handling for calf inspection and navel disinfection was conducted. The calves handling in the studied farm occurred daily from 8 am to 5 pm. Cows that delivered from 5 pm to 4 am remained with their calves longer (from 3h to 12h) undisturbed before the first handling of the calf since the farm handlers did not work overnight. The navel disinfection was conducted by two handlers familiar to the animals and previously trained in a standardized way: (1) the handlers remained still in the entrance of the paddock for 15 s enabling cows to have visual contact and be aware of their presence; (2) handlers walked towards the cow with an equable and non-threatening posture (lowered arms and avoiding eye contact with the cow), approaching laterally at an angle of 45° with the ventrodorsal cow axis; (3) one of the handlers roped the calf with a long rope and brought it closer to the fence while the other handler observed the cows for safety reasons; (4) both handlers crossed the paddock fence to exit the paddock; (5) the handlers drove the calf outside the paddock under the fence using the rope, inspected it and performed the navel disinfection using a commercial antiseptic (Umbicura® - Pecuarista d'Oeste), allowing the cows to have visual contact with her calf; (6) after the navel disinfection the handler removed the rope and drove the calf back to the paddock crossing under the fence.

For the behavioral recording, four periods were considered: (1) 'Pre-calving Period': 6 hours before calving (before the complete expulsion of the calf); (2) 'Post-calving Period': 3 hours after the complete expulsion of the calf; (3) 'First handling': the period of calf handling, including inspection and navel disinfection; (4) 'Post-handling Period': from the completion of navel disinfection to 1 hour later. A total of 310 hours of video recordings were analyzed (10 hours / animal). A single observer recorded the cows' behaviors using focal sampling and continuous observation (23).

At 'Pre-calving Period', the behavioral categories 'moving', 'feeding', and 'body posture' were recorded, as described in Table 1, measured as the percentage of observation time (%). At 'Post-calving' and 'Post-handling' periods, behaviors related to the cow-calf interaction were recorded as described in Table 1, also measured as the percentage of observation time (%). The latencies for the cow to touch her calf (Cow latency), and for the calf to stand on their feet (Calf latency) were recorded in minutes. The following additional information regarding the calving was recorded: *i*) calving period (morning/afternoon/night), *ii*) delivery body posture (standing/ lying down), *iii*) distance of the calving cow from the herd (in meters), and *iv*) calf sex.

Table 1 - Ethogram of Gyr cows' behaviors and their calves in the peripartum period.

Categories	**Description**
	'Pre-calving Period'
Kneeling (oc.[a])	Cow's forelegs bent on the floor and hindlegs erect.
Drinking (oc.)	Cow drinking water in the water bowl.
Grazing (%[a])	Cow taking grass on the ground and showing chewing movement.
Feeding (%)	Cow eating silage and concentrates from the trough. Cow with the head above the trough and showing chewing movement.
Straight spine (%)	Cow standing with all four legs erects and spine straight.
Arched spine (%)	Cow standing with all four legs erect and arched spine.
Moving (%)	Cow walking forward or backward.
Lying down (%)	Lying in lateral or sternal decubitus, with the lower part of the body on the floor and legs stretched or retracted.
	'Post-calving Period' and *'Post-handling Period'*
Cow latency (min.[a])	Period between the complete expulsion of the fetus (calving) until the cow touches the calf for the first time with muzzle and/or tongue.
Calf latency (min.)	Period between the expulsion of the fetus (calving) until the calf stands itself on four legs without falling.
Touching (%)	Cow's tongue or muzzle keeping physical contact with any part of the calf's body.
Not interacting (%)	Cow standing or lying without physical contact and/or without interacting with the calf.
Suckling (%)	Cow standing still while the calf sucks on her teats or makes contact with the teats and/or udder region.
Moving (%)	Cow walking forward or backward.

[a]oc. = occurrences (in number); % = percentage of observation time; min = latency in minutes.
Source: Vicentini et al. (24)

At 'First handling' period, the cow protectiveness was assessed by a single trained observer using the video recordings. A '*Maternal Protection Scoring System'* was assigned, in which scores were attributed to '*Aggressiveness*' (1 to 3), 'A*ttention'* (1 to 3), '*Displacement*' (1 to 5), and '*Agitation*' (1 to 4) according to Ceballos et al.

(19). These scores were then added to compose a single scale the *Maternal Composite Score* (MCS). The sum of the scores for Aggressiveness, Attention, Displacement, and Agitation ranged from 4 (min.) to 11 (max.), generating a MCS from 1 to 8. In addition, a single grade for '*Maternal Protective Behavior*' (MPB) was applied from 1 to 5, in which lower scores were indifferent and less protective cows and higher were more defensive and nervous cows (Table 2).

Table 2 - Maternal Protective Behavior (MPB) score of Gyr cows at the first handling of their calves.

Scores	Descriptions
1	Calm cow; remains standing still.
2	Cow runs away from the handler and leaves the calf alone.
3	Cow shows signs of nervousness; flaps the tail; snorts; vocalizes.
4	Cow stands between the handler and calf with nervousness signs, not allowing the handlers to approach the calf.
5	Cow reacts aggressively, threatening[a] and/or attacking[b] the handler.

[a]Threatens: Stares at the handler with head up or head down; presents continuous head movement and/or displacement towards the handler but does not attack.
[b]Attacks: vigorous displacement towards the handler, followed by physical contact with the fence (usually head-butts). Source: Vicentini et al. (24)

2.3 STATISTICAL ANALYSIS

Descriptive statistics and tests of normality were conducted for all behavioral variables using the PROC Univariate (SAS® Institute, INC., Cary, NC). To evaluate the effect of parity on the cows' behaviors, general linear models were fitted, using the PROC GLM (SAS® Institute, INC., Cary, NC). The behaviors at pre-calving (Grazing, Feeding, Straight spine, Arched spine, Moving, Lying down); post-calving (Cows' latency, Calves' latency, Touching, Not interacting); and post-handling periods (Touching, Not interacting); in addition to MCS and MPB scores were used in the models as dependent variables. The fixed effects of parity (multiparous *vs*. primiparous) and age of the cow (in months) as a covariate with linear effect were included. For variables with non-normal distribution ('Kneeling', 'Drinking', 'Suckling', and 'Moving') the parities were compared using non-parametric statistics (Mann-Whitney test).

A principal component analysis (PCA) was used to investigate the structure of correlation among the behavioral variables at pre-calving, post-calving and post-handling. The behaviors at 'Pre-calving' (Kneeling, Drinking, Grazing, Feeding, Straight spine, Arched spine, Moving, Lying down), ‘Post-calving’ (Cows’ latency, Calves’ latency, Touching, Not interacting, Suckling, Moving), and ‘Post-handling’ periods (Touching, Not interacting, Suckling, Moving) were included in a matrix of animals (*rows*) per behaviors (*columns*). Principal components (PC) with eigenvalues above 1 were retained, and variables with loadings above 0.5 were regarded as the main contributors to the PC.

The Pearson’s correlation coefficient was used to investigate the relationships between the maternal protectiveness scores are the cows’ behaviors at the three periods and the PC obtained in the PCA. In all analyses, *P*-values ≤ 0.05 were regarded as significant, and P-values ≤ 0.10 were discussed as tendencies.

3 RESULTS

3.1 COWS’ BEHAVIORS AT PRE-CALVING, POST-CALVING, AND POST-HANDLING PERIODS

Regarding the calving time, 22.6% occurred in the morning, 45.2% in the afternoon, and 32.2% at night. Most of the cows delivered lying down (90.3%), and only 9.7% delivered standing up. Regarding the calving distance from the herd, 38.7% of the cows calved ‘very close’ (≤ 1 m) to the herd, 12.9% calved ‘close’ (> 1 and ≤ 4 m), 6.5% calved ‘next’ (> 4 and ≤ 6 m), and 41.9% calved ‘away’ (> 6 m) from the herd. For the calf sex, 51.6% were male and 48.4% female (Table 3).

Table 3 - Relative frequency (%) of calving period, calving position, calving distance, and calves' sex of Gyr cows by parity.

	Total	Primiparous	Multiparous
Calving period			
Morning (06:00 a.m. to 11:59 a.m.)	22.6% (7/31)	18.7% (3/16)	26.7% (4/15)
Afternoon (12:00 p. m. to 05:59 p.m.)	45.2% (14/31)	62.6% (10/16)	26.7% (4/15)
Night (06:00 p.m. to 05:59 a.m.)	32.2% (10/31)	18.7% (3/16)	46.6% (7/15)
Calving position			
Lying down	90.3% (28/31)	93.7% (15/16)	86.7% (13/15)
Standing up	9.7% (3/31)	6.3% (1/16)	13.3% (2/15)
Calving distance			
Very close (1 ≤ m)	38.7% (12/31)	37.5% (6/16)	40.0% (6/15)
Close (> 1 and ≤ 4 m)	12.9% (4/31)	12.5% (2/16)	13.3% (2/15)
Next (> 4 and ≤ 6 m)	6.5% (2/31)	6.2% (1/16)	6.7% (1/15)
Away (> 6 m)	41.9% (13/31)	43.8% (7/16)	40.0% (6/15)
Calves' sex			
Male (♂)	51.6% (16/31)	62.5% (10/16)	40.0% (6/15)
Female (♀)	48.4% (15/31)	37.5% (6/16)	60.0% (9/15)

Source: Vicentini et al. (24)

The most prevalent behavioral category at Pre-calving period was standing with 'Straight spine'. Primiparous and multiparous cows differed for the behavior 'Arched spine' ($F = 4.23$; $P = 0.05$) and a tendency was found for 'Moving' ($F = 3.58$; $P = 0.07$). Primiparous cows had three times more standing with 'Arched spine' and tended to move more than the multiparous (Table 3).

At the Post-calving period, the most prevalent category was 'Touching' the calf, and at the Post-handling period, it was 'Not interacting' with the calf (Table 4). Primiparous and multiparous cows did not differ ($P > 0.05$) in the Post-calving and Post-handling behaviors.

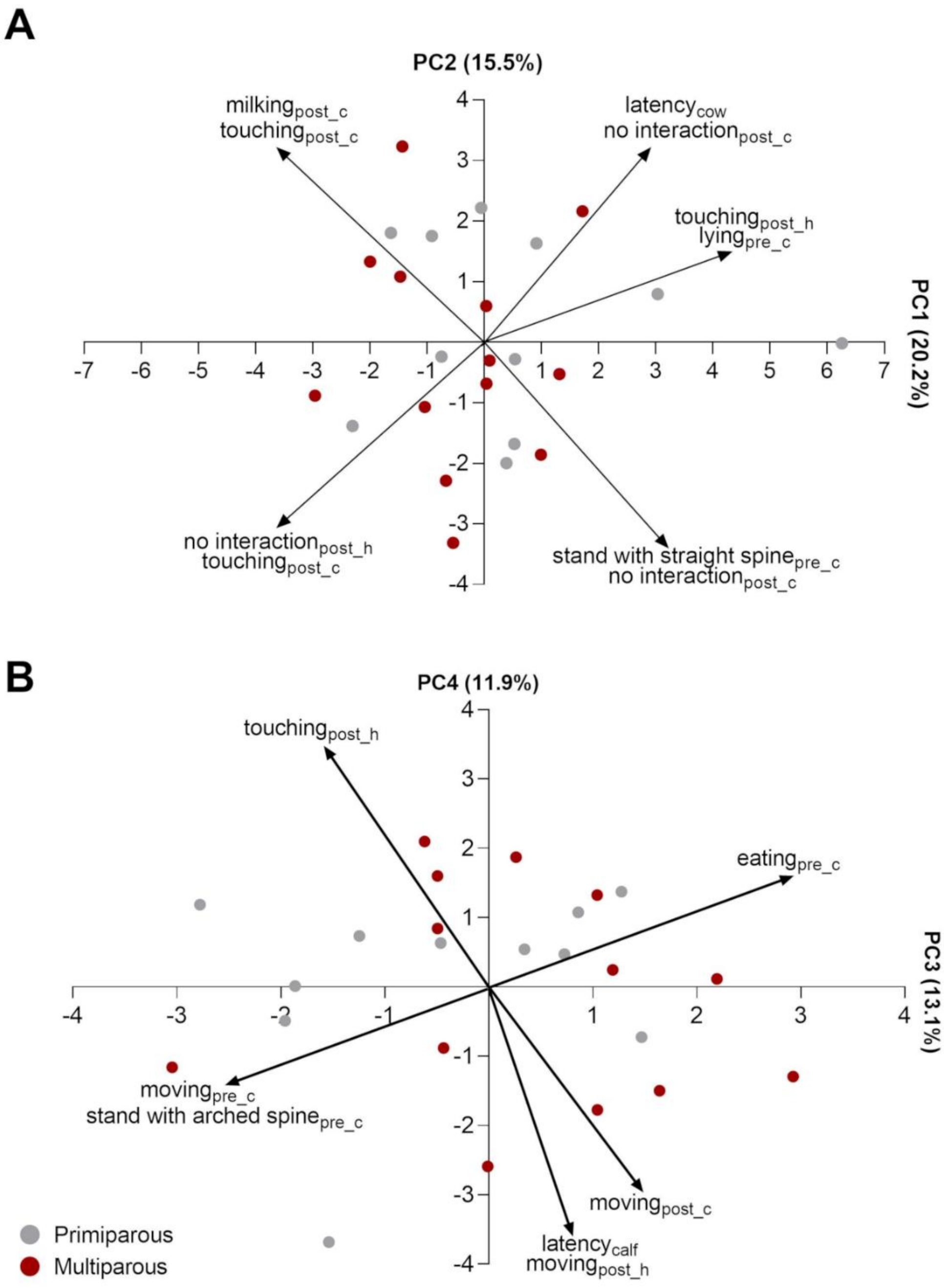
Table 4 - Means (± standard deviation) of Gyr cows' behaviors in the peripartum period.

Cows' behavior	Mean±Std	Primiparous	Multiparous
'Pre-calving Period'			
Kneeling (oc.[a])	1.29±2.81	1.23±3.58	1.35±1.98
Drinking (oc.)	1.40±1.33	1.30±1.25	1.50±1.45
Grazing (%[a])	10.63±13.32	9.23±10.84	15.58±85.46
Feeding (%)	5.45±4.38	5.13±4.41	5.75±4.49
Straight spine (%)	36.97±16.07	32.93±16.63	40.17±15.16
Arched spine (%)	10.65±12.87	15.71±16.00^{A}	5.95±6.77^{B}
Moving (%)	13.57±8.46	16.70±10.16^{A}	10.69±5.40^{B}
Lying down (%)	22.74±13.93	20.34±13.87	24.96±14.11
'Post-calving Period'			
Cows' latency (min.[a])	6.09±19.93	10.81±28.24	1.65±1.76
Calves' latency (min.)	60.61±40.38	68.87±44.66	52.82±35.48
Touching (%)	50.76±16.51	49.39±16.80	52.03±16.72
Not interacting (%)	44.78±17.77	45.64±17.47	44.00±16.62
Suckling (%)	3.51±4.69	4.05±4.52	3.00±4.95
Moving (%)	0.95±2.01	0.93±2.30	0.97±1.78
'Post-handling Period'			
Touching (%)	34.75±19.01	36.87±16.41	32.63±84.49
Not interacting (%)	59.73±19.44	56.46±17.10	62.99±21.58
Suckling (%)	3.44±5.35	3.96±5.86	2.92±4.92
Moving (%)	2.08±6.13	2.71±8.00	1.46±3.59

[a]oc. = number of occurrences; % = relative frequency; min = latency in minutes. $^{A-B}$ Different letters in the same line indicate significance (P ≤ 0.05) or tendency (P ≤ 0.10). Source: Vicentini et al. (24)

In the PCA, four PC had eigenvalues above 1 and, together, explained 60.74% of the total variance in the dataset (Table 4). The PC1 explained 20.24% of the total variance and had higher loadings for 'Lying down' (Pre-calving), 'Cows' latency', 'Not interacting' (Post-calving), and 'Touching' (Post-handling), while higher negative loadings were found for 'Not interacting' (Post-handling) and 'Touching' (Post-calving). This axis might have distinguished cows that spent more time lying down at Pre-calving period, spent more time without interacting with the calf, and took longer to interact with the calf (higher scores in PC1) from those who touched the calf more frequently at Post-calving and less frequently at Post-handling period (lower scores in PC1) (Figure 1A).

Figure 1 – Plot of animals in the PC1 vs. PC2 (A) and PC3 vs. PC4 (B) extracted using behavioral data of Gyr primiparous (gray) and multiparous (red) cows at peripartum period (n = 24).



pre_c = pre-calving, post_c = post-calving and post_h = post-handling periods. Source: Vicentini et al. (24)

The PC2 explained 15.51% of the total variance and had higher positive loadings for ‘Suckling’ (Post-calving) and ‘Touching’ (Post-calving), and negative for ‘Straight spine’ (Pre-calving) and ‘Not interacting’ (Post-calving) (Table 5). This axis ranged from cows that spent more time touching and suckling their calves at Post-calving period (higher scores in PC2), to those who spent more time standing with straight spine at Pre-calving and less time interacting with their calves at Post-calving period (lower scores in PC2), (Figure 1A). Therefore, cows' scores in this PC can be an indicator of the frequency of nursing behavior at Post-calving period.

Table 5 - Principal Components Analysis of Gyr cows and their calves' behaviors in peripartum period.

Behaviors	**PC1**	**PC2**	**PC3**	**PC4**
Cows' latency	**0.711**[a]	-0.011	-0.140	0.097
Calves' latency	0.453	0.133	0.241	**-0.674**
Straight spine $_{pre\_c}$[b]	-0.395	**-0.628**	-0.010	0.378
Feeding $_{pre\_c}$	0.216	-0.462	**0.563**	0.135
Moving $_{pre\_c}$	-0.190	-0.166	**-0.721**	0.010
Lying down $_{pre\_c}$	**0.727**	0.272	0.187	-0.179
Arched spine $_{pre\_c}$	0.287	0.305	**-0.657**	-0.128
Touching $_{post\_c}$[b]	**-0.577**	**0.617**	-0.114	0.152
Not interacting $_{post\_c}$	**0.574**	**-0.733**	0.070	-0.108
Suckling $_{post\_c}$	-0.157	**0.759**	0.235	0.096
Moving $_{post\_c}$	-0.368	-0.097	-0.405	**-0.564**
Touching $_{post\text{-}h}$[b]	**0.561**	0.292	-0.256	**0.644**
Not interacting $_{post\_h}$	**-0.663**	-0.339	0.308	-0.330
Moving $_{post\_h}$	0.207	0.037	-0.334	**-0.708**
Eigenvalue	3.643	2.793	2.364	2.136
Variance explained (%)	20.24	15.51	13.13	11.86

[a]Values in bold represent the higher contributions to each PC (above 0.5); [b]pre_c = pre-calving period; post_c = post-calving period; post_h = post-handling period. Source: Vicentini et al. (24)

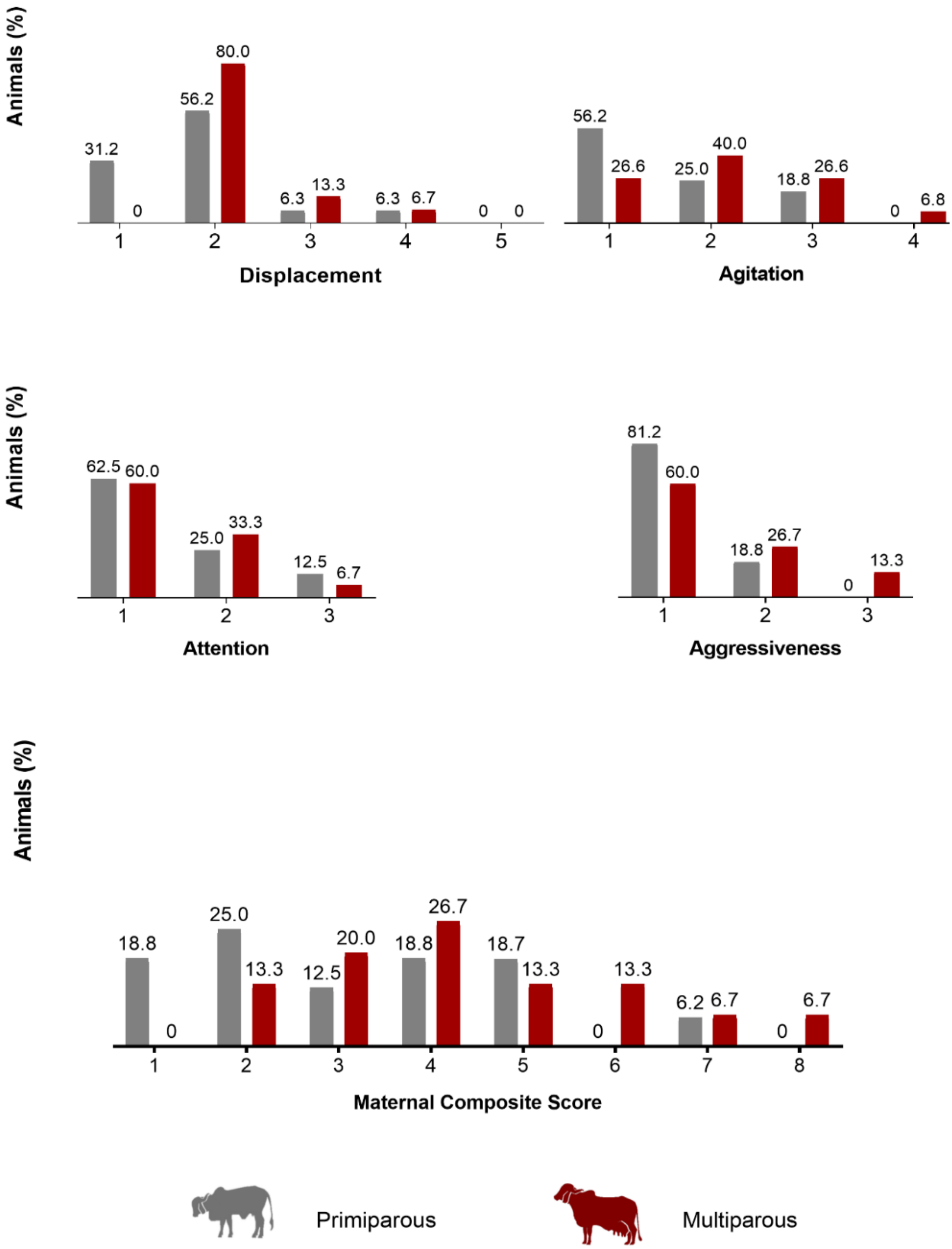
The PC3 explained 13.13% of the variance, showing higher positive loading for 'Feeding' (Pre-calving), and negative loading for standing with 'Arched spine' (Pre-calving) and 'Moving' (Pre-calving) (Table 5). This PC might have reflected the comfort/discomfort of cows at Pre-calving period, ranging from cows that spent more time eating (less evidence of discomfort) to those who spent more time moving and standing with arched spine (more evidence of discomfort) (Figure 1B).

Finally, PC4 (10.70% of variance) had higher positive loading for 'Touching' (Post-handling), and negative for 'Cows' latency', 'Moving' (Post-calving) and 'Moving' (Post-handling). This axis ranged from cows that spent more time touching their calves at Post-handling period, to those who moved more at Post-calving and Post-handling and had longer latency to touch their calves (Figure 1B).

3.2 MATERNAL DEFENSIVENESS

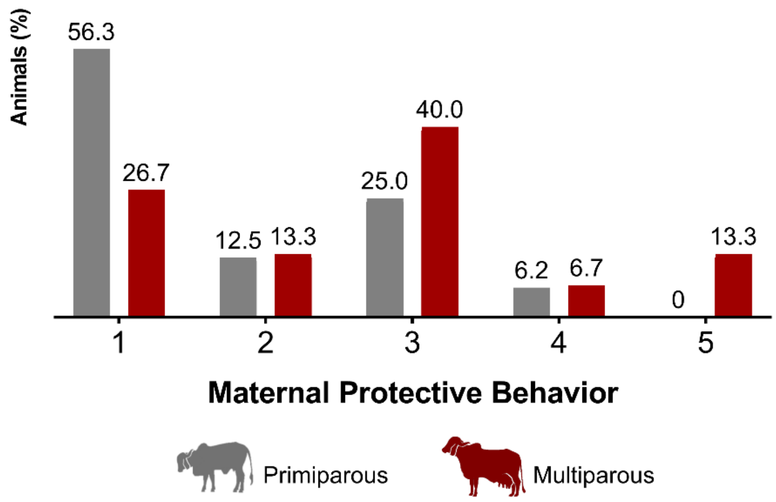
The distributions for the ‘Displacement’, ‘Agitation’, ‘Attention’ and ‘Aggressiveness’ scores and ‘*Maternal Composite Score’* (MCS) are displayed in Figure 2, and ‘*Maternal Protective Behavior*’ (MPB) distribution is shown in Figure 3. The parity showed a tendency on MCS (F =3.57; P = 0.06) and MPB (F = 3.65; P = 0.06) scores. Multiparous cows had higher grades for both scores (4.40±1.76; 3.27±1.79, respectively) than the primiparous cows (3.19±1.76; 2.12±1.50, respectively), indicating that the multiparous tended to be more protective than primiparous cows.

Figure 2 – Maternal Protection Scoring System of primiparous and multiparous Gyr cows at the first handling of their calves (n = 31).



Source: Vicentini et al. (24)

Figure 3 – 'Maternal Protective Behavior' (MPB) of primiparous and multiparous Gyr cows at the first handling of their calves (n = 31).



Source: Vicentini et al. (24)

3.3 RELATIONSHIPS BETWEEN COWS BEHAVIORS AT PERIPARTUM AND MATERNAL DEFENSIVENESS

The maternal defensiveness scores were correlated with cows' behavioral categories. A positive correlation was found between the MPB score and 'Feeding' behavior at Pre-calving (r = 0.425; P = 0.030), indicating that more protective cows spent more time eating in the feeder at Pre-calving period. A positive correlation was also found between 'Displacement' score and 'Moving' behavior Post-calving (r = 0.579; P = 0.008), showing that cows that displaced more during their calves handling also moved more in the Post-calving period. Finally, a negative correlation was found between 'Attention' score and 'Lying down' behavior (r = -0.444; P = 0.023). Cows characterized as less attentive to their calves' handlings spent more time laying down during Pre-calving period.

The maternal defensiveness scores were also correlated with the four PC obtained in the PCA. A negative correlation was found between MPB and 'PC2 (r = -0.457; P = 0.02), showing that cows that spent more time nursing (touching and suckling their calves, with higher scores in PC2), had lower MPB being calmer and less nervous/aggressive during the handling of their calves. In addition, 'PC4' was negatively correlated with 'Displacement' score (r = -0.529; P = 0.07) and positively with 'Attention' score (r = 0.495; P = 0.01). Cows that spent more time touching their calves and had lower latency to touch the calf (higher scores in PC4) tended to move less at Post-handling period and were characterized as more attentive during the first handling of their calves.

4 DISCUSSION

The maternal behavior of cows can influence both the milk production and the performance of their calves, making this a topic of important relevance for the production industry. In addition, issues related to animal welfare and caretakers' safety can also be impacted by the management and behavior of cows in the peripartum period. The objectives of this study were to characterize the behavior of primiparous and multiparous Gyr cows in the peripartum period and its relationship with maternal defense during the first handling of their calves. Parity was related to both peripartum behavior and maternal protection. Primiparous cows showed more signs of pain and discomfort during the prepartum period than multiparous cows. Both primiparous and multiparous were protective, but only multiparous cows were aggressive ones. The peripartum behavior and maternal protectiveness were also related. The most protective cows ate for the most time in the prepartum period, while the least attentive were the ones that spent more time lying down in the prepartum period. The cows that nursed, stimulated, and touched their calves more frequently were also calmer and more attentive.

The higher incidence of daytime calvings (morning and afternoon) may be related to the selective advantages of calving at different times of the day or may even result from conditioning the animals to the farm routine (6). Among the factors that can influence the period of calving, Proudfoot et al. (25) highlighted changes in light patterns, diurnal hormones, and management routine. Regarding calving position, the vast majority of cows calved lying down, corroborating what is already described in the literature as the most frequent calving position by cows (26). The position of calving must be taken into account as an important practical factor and indicator of difficulties in the calving process. Albeit zebu cattle show a lower frequency of dystocia, the cows' posture during parturition may indicate obstetric problems, from which there is a greater risk of calf death when the cow gives birth standing up (27, 28). Regarding the calving distance, cows usually tend to move away from the herd in the early hours before calving and looking for a quieter and hidden place. This distancing behavior has an adaptive value that may be preserved in domestic species, avoiding the risk of offspring death by predators and other threats (2). However, we emphasize that this behavior, whether moving away from the herd (separation behavior) or not (aggregation behavior), is a phenotype with plasticity potential influenced by several factors (3, 25, 29). In cattle and other domestic ungulate species, calving females can only distance themselves from the herd when the environment is favorable (*e.g.,* presence of shelter, dense and natural vegetation, topography condition); otherwise, they calve in the herd (2, 25, 30). In our study, the maternity paddock had no shelter or natural vegetation, which may have led the cows to calve closer to each other. The size of the maternity paddock (smaller than pasture areas) and proximity to management facilities (with a high frequency of traffic of working machinery and people) may also be related to a higher incidence of calving cows close to the herd in this study.

At the final gestation period, both fetal growth increase and energetic mobilization by the fetus can influence the behavior of cows, promoting the reduction of feed intake and movements. In the hours before calving, cows become more restless (6, 31). The higher frequencies of feeding behavior and resting behavior compared to other behaviors may be related to physiological changes prior to the calving. Cows tend to decrease their food and water consumption before giving birth, but not completely. In the study by Jensen (32), Holstein cows decreased but did not stop water and food intake in the hours before calving. The rupture of the amniotic sac seems to be responsible for stimulating consumption since it relieves pressure in the abdominal region of cows (32, 33). In relation to moving behavior, previous studies both in European cattle (32, 34-37) and Zebu cattle (38), demonstrated that in the hours before calving cows tend to move more. Huzzey et al. (34) observed an increase of standing bouts of Holstein cows housed in free-stall systems during the calving day using pedometer devices. Using behavioral observation by video recordings, Miedema et al. (31) and Jensen (32) described increased frequency of lying bouts of Holstein Frisian cows kept indoors six hours before calving. In Holstein cows kept on pasture, Rice et al. (39) found an increase of lying bouts between three and four hours before calving through pedometers. In a similar field condition to the present study, using intra-ruminal transponders, a previous study with Gyr cows showed an increase of activity 11 hours before calving (38).

The increased activity and movements can be related to pain and discomfort, signs from myometrial contractions, and the fetus expulsion (6, 40). In our study, primiparous cows moved more and spent more time with an arched spine, which we understand as signs that show more effort and discomfort in the parturition process than multiparous cows. Cattle are known to arch their spines under physiological and pathological situations. During the delivery process (40), vaginal exams (41), and in severe cases of laminitis (42), the arched spine seems to be directly related to pain and discomfort. There are also anatomical and physiological differences between age, and consequently, parity (*e.g.*, organ size, shape, cervical dilation) which contributes to facilitating the calving process for multiparous cows, who showed fewer discomfort signs in the present study (26, 32).

The first component of PCA (PC1) revealed variables related to the maternal investigation. After calving, the cow's attention tends to be directed towards the newborn immediately, and a strong cow-calf bond is established (6, 7). Cows that take longer to touch their calves can compromise the quality of this bond. Cows with higher scores in PC1 spent more time lying down in the Pre-calving period and stayed longer time with no interaction, taking longer to touch their calves for the first time. We could infer that the relationship between cows that spent more time lying down in the Prepartum period with the greatest latency in touching their calves may be due to exhaustion from the labor process. Edwards et al. (26) observed an association between cow exhaustion and delay in standing up soon after calving. Other factors, like environmental (*e.g.,* presence of predators, weather conditions) and physiological (*e.g.,* calf weight, calves' vigor) conditions, can influence the time of calf investigation and stimulation by the dam (27, 43, 44). In the second PC (PC2), variables with higher loadings were those related to maternal nursing and stimulation. Good quality of stimulation and maternal care in the early hours of life ensure the survival and good performance of the calves (44). In PC2, cows that touch their calves more frequently also suckle the calf longer and sooner after calving, showing higher scores in PC2. In addition to maternal care, the success of the first suckling is crucial for offspring survival (45). Schmidek et al. (46) state that the calf's first suckling should occur within the early 3 hours of life. Therefore, cows that suckled the calf earlier and longer can be considered as having better maternal performance.

In turn, PC3 reflected variables related to comfort/discomfort of cows in the Pre-calving period, ranging from cows that spent more time feeding at the Pre-calving period to those who spent more time moving with arched spine. As previously discussed, the movement and arched spine posture might indicate pain and distress in the calving process. Mainau et al. (40) attribute these pain signs to physiological alterations caused by calving, the widening of the cervix's and accentuated myometrial contractions. On the other hand, the feed intake behavior may be related to the absence of severe pain or relief of abdominal pressure by rupture of the amniotic sac (33).

The variables in PC4 also reflected the maternal nursing behaviors, ranging from cows that spent more time touching their calves after handling to those who spent more time moving and showed higher latency to touch their calves after birth. The cows can perceive their calves' first handling as a potential threat (14). After the reunion of cow and calf after handling, it is natural for the cow to lick and smell the calf, investigating it. Both cows (47), goats (48), and ewes (49) are known to lick their offspring after a separation period. Animals that moved more during the post-calving and post-handling periods may have tried to distance themselves from other cows and handlers in an attempt to protect the calf. Cows that are more frightened and perceive threats around them spend more time in vigilance, and this can result in negative effects on the latencies to stand up and first suckling, taking longer time to touch their calves after birth (27, 45).

The investigation, stimulation, and nursing are components (traits) of maternal behavior that play an important role in the calves' health and safety. Similarly, maternal protection also assists in better chances of offspring survival (12). Regarding maternal defense, our results showed that both multiparous and primiparous Gyr cows tend to move less and be more attentive during the handling of their calves. However, multiparous cows tended to have higher scores for agitation and aggressiveness than primiparous ones. These results suggest two main styles for the dams who were characterized as defensive in Gyr cows: 'Protective-attentive mothers' and 'Protective-aggressive mothers'. Along these lines, in our study those cows defined as 'Protective-attentive mothers' were the cows that were alert and attentive during calves' handling but did not threaten or attach the handlers. In turn, 'Protective-aggressive mothers' were those cows who were attentive and hostile (threatening and/or attacking) during their calves' handling. The expression and intensity of maternal defense may reflect several individual factors such as temperament, body condition, and sex and vigor of offspring, in addition to previous experience and parity that seem to influence this behavior (11, 26, 44, 50, 51). In taurine cattle, previous studies also showed that multiparous cows were more protective than primiparous ones when their calves were handled (9, 13, 18).

To our knowledge, there have been no previous reports evaluating maternal defensive behavior related to parity in zebu cattle; however, some studies have been conducted evaluating other aspects of maternal defensiveness in this subspecies. According to Pérez-Torres et al. (14), Gyr and Brahmans cows showed higher intensity of maternal protection until 90 days postpartum. For these animals, offspring protection seems to be so important that cows defended both their own and other cows' calves (14). In studies with Gyr and Brahman cows, Orihuela et al. (17) found no relationship between temperament and maternal defense in the peripartum period. However, cows more reactive to calves' handling were those with more aggressive behavior toward humans (17). Ceballos et al. (19) investigated maternal protectiveness in Holstein/Gyr crossed cows, reporting that the aggressive cows were also more frightened, irritated, and agitated during handling. Zebu cattle are widely known to be more reactive to handling than European cattle (22, 52, 53), and all these findings may suggest that more excitable behavior can also be seen in terms of maternal defense in some cows. Our results also indicate that the exacerbated defensiveness of multiparous Gyr cows observed in this study might suggest that even animals habituated to handling routines can react strongly to the handling of their newborn calves (5, 9). The newborn care practices are essential for their health (*e.g.* navel asepsis, antiparasitic medicine, suckling assistance) but require close contact between the calving cows and the handlers (5, 44). So, the aggressive cows may be a severe one-welfare problem, increasing the risk of stress and labor accidents for both handlers and animals.

The correlations showed a relationship between pre-calving and post-calving behaviors with maternal defense. The most defensive cows were the ones that spent more time feeding in the pre-calving period. Stěhulová et al. (51) reported that cows in better body conditions are more protective with their calves, demonstrating a relationship between feed-intake and maternal defense. Furthermore, these animals with greater feed-intake were also those showing fewer signs of pain and discomfort during the calving process, suggesting that cows without signs of severe pain or less weariness are those that defend their calves more. Edwards et al. (26) reported the influence of exhaustion on delayed standing after calving, which can, in part, explain the relationship found between weariness and defensive behavior in Gyr cows in this study.

A correlation was also found between ‘Displacement’ score during the calves' handling and the time spending moving after calves’ handling. This behavior may reflect the cows’ disturbance caused by their calves' handling. Some cows may perceive the caretakers as a threat, and this displacement behavior during the handling can result from a nervous emotional state (14, 19). We attributed the moving behavior after handling as an evasive strategy to move away with their calves from this perceived threat. The ‘Attention’ score was correlated with ‘Lying’ behavior during pre-calving, so cows less attentive to the handling of their calves spent more time lying down in the pre-calving period. The lying down position is related to the final stages of calving however can also be related to weariness, as discussed above (26). Therefore, the less attentive cows may have been wearier due to the strain of the calving process.

The MPB was negatively correlated to PC2, indicating that higher scores in PC2 (cows spending more time nursing and touching their calves) had lower defensiveness scores (calmer and non-aggressive during the calves’ handling). Maternal protectiveness is positive and beneficial in the herds in which calves and cows are kept together. It is important that the dam licks and stimulates the offspring, facilitating recognition and contributing to a strong bond (54-56). Likewise, it is also important that cows do not attack caretakers. In the practical context, desirable cows in the herd are those with good maternal ability that nurture and protect the calf and accept their handling.

Similarly, the correlation between MPB and PC4 showed that cows with better maternal nursing behaviors moved less and were more attentive to the handling of their calves. These results indicate that cows with better maternal performance (lower latency in touching the calf after calving, suckling longer, and touched their calves more) also had better maternal temperament. Thus, we could infer that cows labeled as 'Protective-attentive mothers' and presenting a lower risk of danger to the handlers were better mothers than 'Protective-aggressive mothers' cows. Our results indicated that both multiparous and primiparous cows were protective, but only multiparous cows were regarded as aggressive. While these findings suggest that primiparous cows did not present any aggressive behavior towards caretakers, it is not clear if it was a result of weariness from the calving process (more intense in primiparous) or if perhaps there was some stimulus during the calf management process that had triggered the aggressive behavior in multiparous cows. Our results also bring a new perspective on maternal defense behavior in zebu cattle, highlighting the implications of cow behavior in the peripartum period. Further studies to better understand maternal aggressiveness and the factors that influence it may enhance the management efficiency in dairy farms and cow-calf operations, ensuring the safety of handlers and caretakers.

5 CONCLUSIONS

In conclusion, the peripartum behaviors of both primiparous and multiparous Gyr cows are related to the dam interactions with the calf and maternal defensiveness. Primiparous cows showed more behavioral signs of pain and discomfort during prepartum, which may have affected their interaction with their calves. Multiparous cows showed less behavior indicative of pain and discomfort during the parturition process. Both primiparous and multiparous cows tended to be protective, but only multiparous cows showed aggressive behavior towards the caretakers. The most protective cows spent more time feeding, while less attentive cows spent more time lying down during the pre-calving period. Cows with better maternal performance (nursing, stimulation and touching their calves) were calmer, moved less, and were more attentive during the handling of their calves.

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5 CHAPTER III

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Effect of training to first milking involving positive tactile stimulation on defense and maternal care at post-calving period in primiparous Gyr dairy cows

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ABSTRACT: In dairy systems with Zebu breeds, calves are not immediately separated from their dams after calving, so maternal care and protective behavior are important, influencing both productive performance and stockpeople safety. Our objectives were to: 1) investigate the effects of a training protocol involving pre-calving positive stimulation, delivered prior to calving, on maternal care of primiparous Gyr cows; and 2) evaluate the effects of this training protocol on maternal protective behavior towards handlers during the first calf handling. Primiparous dairy Gyr cows (n = 37) were allocated into two groups: Training (n = 16) and Control (n = 21). Animal behaviors were recorded in three periods: post-calving, first calf handling, and post-handling. Maternal protective behavior during calf handling was accessed using measures of aggressiveness, attention, displacement and agitation. Calf latency to stand up ($P < 0.01$), weight ($P = 0.07$) and sex ($P < 0.01$) differed between training and control groups. The Training group had less touching ($P = 0.03$), more time not interacting with the calf ($P = 0.03$), tended to be less protective ($P = 0.056$) and moved less ($P < 0.01$) during the first handling of their calves. In conclusion, primiparous dairy Gyr cows submitted to pre-calving training protocol were calmer on the first handling of their calves and had less maternal care.

Keywords: aggressiveness, cow-calf contact systems, Zebu

1 INTRODUCTION

Improving human-animal interactions addresses worldwide societal demands for better animal welfare, an impetus for improvement in diverse animal production fields involving animal handling [1,2]. The quality of human-animal interaction is an important factor that affects the welfare of several dairy species, such as cattle, buffaloes, sheep, and goats (for a review see Napolitano et al., [3]). In dairies, the demand for improving human-animal relationship is evident as animals are handled daily for milking and have direct contact with handlers [4,5].

There are various ways to improve animal handling and consequently animal welfare, including selection of handlers [6], improving facilities [7,8], or using gentle handling procedures [9-11]. Gentle handling, e.g., stroking body regions, gentle tactile stimulation, and vocal interactions in European dairy cattle (*Bos taurus taurus*) improved animal responses to humans and handling routines [10]. Stroking body regions of Brown Swiss and Austrian Simmental lactating cows, Schmied et al. [12] reported that gentle stimulation reduced avoidance distance and increased approaches to humans. Lürzel et al. [10] reported that gentle tactile stimulation and vocal interactions with Holstein-Friesian heifers induced behavioral responses compatible with pleasure during stroking. In Gyr (*Bos taurus indicus*) dairy cattle, pre-calving brushing stimulation reduced cows' reactivity score, respiratory rate and rectal temperature [13]. Similarly, primiparous and multiparous Gyr cattle subjected to positive tactile stimulation in the prepartum period reduced reactivity during milking, such as stepping and kicking [14]. The training protocol was more efficient in primiparous versus multiparous cattle to maintain adequate behaviors and milk ejection during their first milking [14]. Paranhos da Costa, et al. [15] reported beneficial effects of a training protocol on Hostein-Gyr dairy heifers reducing reactivity during first milking and facilitating the milking routine. These results demonstrated strategies to address management challenges during milking. However, the most challenging handling procedures for a dairy cow may start immediately after calving.

On tropical dairy farms, heifers are usually infrequently handled and maintained in pasture-based systems before first calving and when handling procedures occurs, they are generally aversive or neutral (e.g., vaccination, hot-iron branding, artificial insemination, weighing). This less-frequent and generally aversive interaction can lead to greater fear and consequently reactive responses to human presence and handling procedures [16,17]. It can be aggravated in the peripartum period, which is very sensitive for both dam and calf, further complicating management. In dairy farms with European breeds, calves are usually separated from their dams within the first 24 hours of life [18,19]. However, on most pasture-based farms with Zebu dairy breeds, separation of dam and calf is not performed, as it may compromise lactation persistence [20,21]. Thus, the first handling of dams and calves after calving can be challenging for Zebu dairy cattle. Handling newborn calves is necessary for health care, including navel asepsis, weighing, and identification [22]; however, these procedures can be hampered by the dam's presence [23]. Cows that perceive these interventions as a potential threat to their calf may exhibit extreme protective behaviors leading to a high risk of accidents to stockpeople and calves [24,25]. Issues related to behavior and handling of recently calved cows are relevant in the livestock industry because aggressive cows can compromise one-welfare and farm sustainability [23,24,26,27].

In this context, positive handling could be used in day-to-day farm management to improve the welfare and safety of animals and handlers [9-11,14]. Perhaps a previous positive experience (e.g., habituation to humans and gentle handling) mitigates aggressive reactions of recently calved cows toward humans [23]. However, to our knowledge, no studies have evaluated effects of gentle tactile stimulation protocols before calving on maternal care and protective behavior of Zebu dairy cows in cow-calf contact systems.

Thus, using primiparous Gyr dairy cows, we wanted to determine the effects of a training protocol for milking, involving gentle tactile stimulation prior to calving, on: 1) maternal care during the post-calving period; and 2) maternal protective behavior towards handlers during the first handling of calves. We hypothesized that 1) the training protocol would affect the cows' maternal behavior; and 2) cows subjected to the training protocol would have lower protective behavior against stockpeople during the first handling of their calves..

2 MATERIAL AND METHODS

This study was approved by the Ethics Committee of Animal Use of the Instituto de Zootecnia, Nova Odessa, São Paulo, Brazil (CEUA/IZ 230-16).

2.1 ANIMALS AND HANDLING

Thirty-seven primiparous Gyr dairy cows (*Bos taurus indicus*), aged 46.26 ± 8.63 months, from an experimental farm station (Empresa de Pesquisa Agropecuária de Minas Gerais – Epamig Oeste, Uberaba, Minas Gerais State, Brazil) were used. Only eutocic calvings of singleton births between August and December 2017 were included. A pasture-based system was used for all cattle. Cows were allocated into two groups: Training (n = 16) and Control (n = 21), based on the expected calving date (the first animal to calve was randomly allocated to one group, the next cow to calve was then allocated into the alternate group, with this pattern continuing for the remaining cows). In the Training group, 40 days before the estimated calving day, cows started a training protocol for the first milking, involving gentle tactile stimulation (14 days consecutively) in a conventional tandem parlor (12 milking machines, in two rows). The protocol was performed by six trained handlers and constituted three phases, as described by Ujita et al. [14]. Briefly, in Phase 1 cows were driven from the pasture to the milking parlor and passed through the milking stalls; Phase 2, cows went through the milking stalls and were brushed (2 min.) on the whole body (head, neck, trunk, udder, front legs, and hind legs); and Phase 3, cows went through the milking stalls, their body (mainly udder and hind legs) was brushed (2 min.), legs restrained and teat asepsis (pre-dipping) was performed. Additional information about the milking parlor and milking routine is described in Ujita et al. [14]. The Control group did not receive the training protocol and was subjected to the normal farm management of the farm. Thirty days before the expected calving day, cows of both groups were relocated from the pasture to a maternity paddock (0.55 ha; maximum stocking density: ~27 cows/ha), with both Control and Training group cows kept in the same paddock. Nutritional management of the animals of both groups in the maternity consisted of corn silage and 500 g concentrate per animal, delivered to the feeder twice a day using a tractor vehicle. Additionally, water and mineral salt were offered ad libitum. The animals weighed 425.5 ± 47.3 kg (Training group: 427.5 Kg ± 37.1 kg; Control group: 422.5 ± 37.1 kg). Details about the maternity paddock and nutritional management of cows are fully described in Vicentini et al. [28]. During final gestation period, cows were not handled or disturbed (except for the Training group during the protocol). All routine procedures (i.e., feed delivery, calf weighing and navel asepsis) were performed by the same six handlers trained in good practices of cattle handling.

2.2 BEHAVIORAL OBSERVATIONS

The maternity paddock was equipped with four video monitoring cameras (GIGA, GSHDP20TB) that recorded cow behavior 24 hours a day. Cows were individually identified with non-toxic paint (Koleston, Wella®, Darmstadt, Germany) on both sides of their body. Calving was defined as complete expulsion of the calf. Thereafter, a minimum of 3 hours were allowed for the cow-calf dyad to remain together without any human intervention, with the first calf's handling performed afterward. Calf handling was done daily during handlers working hours (8 am to 5 pm.), cows that calved earlier or later on (5 pm to 4 am) remained with their calves for a longer undisturbed period of time (3 to 15 hours).

Three periods were considered to record maternal behaviors: (1) 'Post-calving period': 3 hours following the complete expulsion of the calf; (2) 'First calf handling': the period of calf handling, including inspection and navel asepsis; (3) 'Post-handling period': 1 hour after the completion of navel asepsis. After 'Post-handling period' both cow and calf were removed from the maternity pen. Based on the video recordings obtained by the monitoring cameras, 189 hours (4.5 hours/cow) were analyzed. A single trained observer recorded cow behaviors using focal sampling and continuous observation [29]. During the 'Post-calving' period, the latency of the first calf touch by the cow ('Cow latency'), and the latency of the calf to stand on its four feet ('Calf latency’) were recorded in minutes (Table 1). Unsuccessful attempts by calves to stand until they were able to stand up without falling (‘Calf attempts’) were recorded as number of occurrences (Table 1). Behaviors related to the cow-calf interaction (‘Touching’, Not interacting’, and ‘Suckling’) in both ‘Post-calving’ and ‘Post-handling’ periods were recorded as the percentage of observation time (Table 1).

Table 1 - Ethogram of Gyr dairy cows’ behaviors and their calves in the Post-calving and Post-handling period.

Categories	Description
Cow latency (min.[a])	Period between the complete expulsion of the fetus (calving) until the cow touches the calf for the first time with the muzzle and/or tongue.
Calf latency (min.)	Period between the expulsion of the fetus (calving) until the calf stands on four legs without falling.
Calf attempts (freq[b])	Number of calf unsuccessful attemps to stand.
Touching (%[c])	Cow’s tongue or muzzle keeps physical contact with any part of the calf's body.
Not interacting (%)	Cow standing or lying without physical contact and/or without interacting with the calf.
Suckling (%)	Cow standing still while the calf sucks on her teats or makes contact with the teats and/or udder region.
Moving (%)	Cow walking forward or backward.

[a]min = latency in minutes; [b]freq. = frequency (in numbers); [c]% = percentage of observation time.
Source: Personal Archive.

In the ‘first handling’ period, cow protective maternal behavior was assessed by a single trained observer using two scoring systems, ‘Maternal Composite Score (MCS)’ and ‘Maternal Protective Behavior (MPB)’, as described by Vicentini et al. [30]. The MCS was obtained by adding the scores of four main behaviors: ‘Aggressiveness’ (1 to 3), ‘Attention’ (1 to 3), ‘Displacement’ (1 to 5), and ‘Agitation’ (1 to 4) [31]. Sum of MCS scores ranged from 4 (minimum) to 10 (maximum), subsequently transformed from 1 to 7. Based on MCS scoring, cows were labeled as 'indifferent' (1-2), 'protective-attentive' (3-5) and 'protective-aggressive' (6-7). The MPB was applied scoring from 1 (calm cows) to 5 (aggressive cows) [30]. Navel disinfection was conducted by two familiar handlers that worked in pairs. Handlers’ approach was standardized, aiming to be consistent and avoid influencing cows’ behavior, as described by Vicentini et al. [30]. After ‘Post-handling period, both cow and calf were moved from the maternity paddock to the corral where colostrum milking and calf identification procedures took place and the calf was weighed (Digitron Scale).

2.3 STATISTICAL ANALYSIS

Descriptive statistics and tests of normality were conducted for all behavioral variables using PROC Univariate of Statistical Analysis System (SAS® Institute, INC., Cary, NC). To evaluate effects of Treatment (Training group vs Control group) on cow and calf behaviors, and maternal protection scores, general linear models were fitted, using PROC GLIMMIX of SAS and adopting the lognormal distribution for variables with non-normal distribution (‘Cow latency’, ‘Calf latency’, ‘Calf attempts’, MCS and MPB). Cows’ behaviors (‘Cow latency’, ‘Touching’, ‘Not interacting’, and ‘Suckling’) and calf behaviors (‘Calf latency’ and ‘Calf attempts’) at ‘Post-calving’ and ‘Post-handling’ periods, and maternal protection scores (MCS and MPB) were used as dependent variables. Treatment (Training group vs. Control group), ‘Calf sex’ (male or female), treatment and calf sex interaction were used as fixed effects. ‘Calf weight’ (in kilograms), and cow age (in months) were included as covariates with linear effect.

Complementary, Chi-square tests in contingency tables were used to estimate as-sociations between Treatment (Training or Control groups) with scores for Aggres-siveness, Attention, Displacement, and Agitation. Pearsons’ correlation coefficients were used to investigate relationships between cow and calf behaviors in ‘Post-calving’ and ‘Post-handling’ periods. P-values ≤ 0.05 were considered significant, and P-values ≤ 0.10 tendencies.

3 RESULTS

There were more male (n = 20) than female (n = 17) calves, and males were heavi-er (Table 2). There were effects of ‘Calf sex’ (F = 9.94; P < 0.01) and ‘Calf sex’ * ‘Calf weight’ interaction (F = 6.97; P = 0.01) on ‘Calf attempts’ to stand up. Male calves al-most double the number of attempts than females (Table 2).

Table 2. Means (± standard error) of primiparous Gyr dairy cow behaviors and calf sex, behavior, and weight in Post-calving and Post-handling periods.

Categories	Mean±SE	Treatments	
		Training	Control
Calf sex (freq.[1])			
Male (♂)	.	11	9
Female (♀)	.	6	11
Calf weight (kg[2])	22.70±0.52	23.62±0.91	22.05±0.61
Male (♂)	23.29±0.72[a*]	23.72±1.22	22.92±0.87
Female (♀)	21.87±0.73[b*]	23.42±1.43[A*]	21.02±0.76[B*]
Calf latency (min.[3])	62.51±7.51	68.87±11.16	57.66±10.25
Male (♂)	68.28±11.18	73.80±15.77	63.27±16.38
Female (♀)	54.93±9.38	60.66±15.13	51.50±12.44
Calf attempts (freq.)	13.54±1.00	14.06±1.47	13.14±1.38
Male (♂)	16.28±1.38[a**]	15.80±1.93	16.72±2.05
Female (♀)	9.93±0.82[b**]	11.16±1.83	9.20±0.72
		'Post-calving Period'	
Cow latency (min.)	4.60±0.81	4.75±1.54	4.50±0.91
Male (♂)	5.30±1.24	5.40±2.21	5.23±1.49
Female (♀)	3.64±0.89	3.66±2.01	3.63±0.92
Touching (%[4])	52.95±2.81	49.38±4.48	55.72±3.56
Male (♂)	48.92±18.36	42.96±4.87[B,b**]	55.63±7.32[A**]
Female (♀)	57.51±11.59	60.95±6.77[a**]	55.80±3.11
Not interacting (%)	42.00±2.87	45.63±4.66	39.17±3.57
Male (♂)	47.43±4.49	53.12±5.07[A,a**]	41.03±7.33[B**]
Female (♀)	35.85±2.81	32.15±5.91[b**]	37.69±3.08
Suckling (%)	3.94±0.90	4.04±1.20	3.85±1.32
Male (♂)	2.90±3.78	3.39±1.57	2.36±0.90
Female (♀)	5.11±6.18	5.22±1.95	5.05±2.25
		'Post-handling Period'	
Touching (%)	38.91±2.99	36.87±4.10	40.53±4.33
Male (♂)	39.73±4.36	38.00±5.76	41.66±6.91
Female (♀)	37.98±4.18	35.00±5.80	39.61±5.77
Not interacting (%)	56.41±3.09	56.45±4.27	56.37±4.50
Male (♂)	53.50±4.53	54.00±6.25	52.96±6.98
Female (♀)	59.66±4.16	60.55±4.84	59.17±6.03
Suckling (%)	3.47±0.94	3.95±1.46	3.08±1.26
Male (♂)	4.47±1.40	3.66±1.69	5.37±2.35
Female (♀)	2.35±1.23	4.44±2.90	1.21±1.15

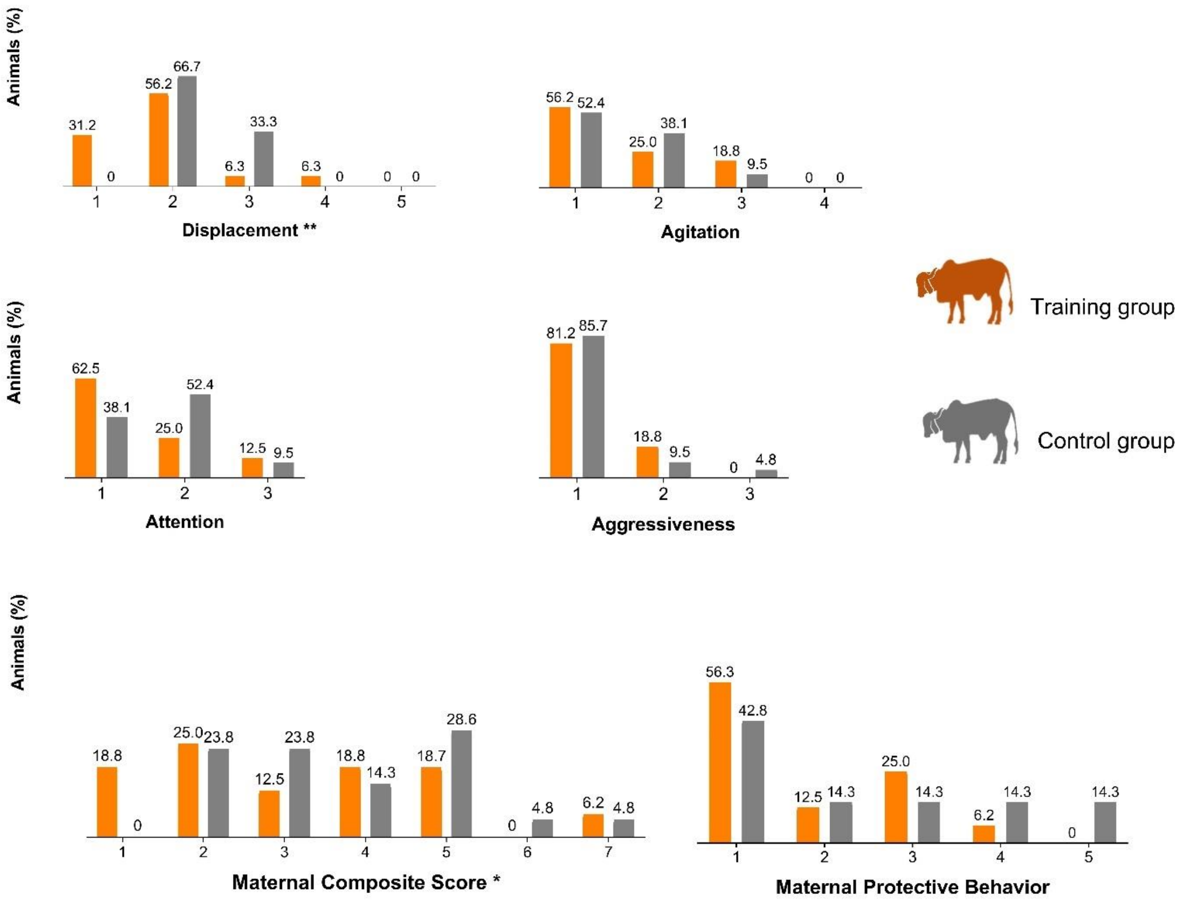
[1]freq. = frequency (in number); [2]kg: kilograms; [3]min = latency in minutes; [4]% = relative frequency; [a – b] Different lowercase letters in the same column of each category indicate significance (P ≤ 0.05)** or tendency (P ≤ 0.10)*; [A – B] Different uppercase letters in the same row of each category indicate significance (P ≤ 0.05) or tendency (P ≤ 0.10). Source: Personal Archive.

Regarding cow behaviors, ‘Calf weight’ ($F = 3.33$; $P = 0.07$) tended to affect ‘Cow latency’, with heavier calves taking longer to be touched by their dams. There were relationships between cow and calf behaviors. A positive correlation between 'Calf latency' and ‘Calf attempts' ($r = 0.63$; $P < 0.01$) indicated that calves with the longest time to stand up were also those that made more attempts to do so. In addition, a positive correlation between 'Calf latency' with 'Not interacting' in both ‘Post-calving’ ($r= 0.36$; $P = 0.03$) and ‘Post-handling’ ($r = 0.41$; $P = 0.01$) periods was detected. Finally, a tendency between 'Calf attempts' and 'Touching' ($r = -0.30$; $P = 0.09$), and a significant correlation between 'Touching' and 'Not interacting' ($r = -0.94$; $P < 0.01$), both at ‘Post-calving period’ were identified.

Regarding effects of training protocol on maternal behaviors in the ‘Post-calving period’, there was a significant effect of Treatment * ‘Calf sex’ interaction on the variable ‘Touching’ ($F = 4.79$; $P = 0.03$). Male calves were less touched than females by cows belonging to the Training group, but there was no difference in the Control group related to calf sex (Table 2). Similarly, there was an interaction between Treatment * ‘Calf sex’ ($F = 4.85$; $P = 0.03$) in 'Not interacting' behavior at ‘Post-calving period’. Training group cows spent more time ‘Not interacting’ with their male calves than mothers of female calves in the same group. In addition, Training group cows spent more time ‘Not interacting’ with their male calves than those from Control group (Table 2). In ‘Post-handling period’, there was an effect of ‘Calf weight’ on 'Touching' behavior ($F = 3.96$; $P = 0.05$), in which heavier calves were touched longer by their dams. Cows from Training and Control groups did not differ in other assessed behaviors.

Distributions of ‘Displacement’, ‘Agitation’, ‘Attention’, ‘Aggressiveness’ scores, ‘Maternal Composite Score’ (MCS), and ‘Maternal Protective Behavior’ (MPB) are presented in Figure 1. Treatment had a tendency on MCS ($F = 3.92$; $P = 0.056$) and an effect on ‘Displacement’ ($F = 10.05$; $P < 0.01$) scores. Control group had higher proportion of animals with higher Displacement scores and a tendency for higher MCS score. Chi-square test revealed an association between Treatment with ‘Displacement’ score, with higher percentage of score 0 for the Training group and a higher percentage of score 3 for the Control group ($\chi^2 = 11.11$; $P < 0.01$).

Figure 1 – Displacement, Agitation, Attention and Aggressiveness scores, Maternal Composite Score (MCS), and Maternal Protective Behavior (MPB) of primiparous Gyr dairy cows at the First handling of their calves.



*Single asterisk indicates tendency (P ≤ 0.10); **Double asterisk indicates significance (P ≤ 0.05). Source: Personal Archive

4 DISCUSSION

Maternal behavior is an important trait in many domestic species [32]. In Zebu breeds this characteristic is even more relevant as it impacts both dam productivity and calf performance [25,33]. The maternal protective behavior provided by a Zebu dam can be a problem for human safety in particular behaviors related to calf defensiveness. Thus, strategies that mitigate exacerbated maternal protectiveness reactions improve the safety and welfare of stockpeople and animals. Our objectives were to investigate effects of a pre-calving training protocol involving gentle tactile stimulation on maternal care and protective behavior of primiparous Gyr dairy cows during the post-calving period. The training protocol was associated with various cow behaviors and maternal protectiveness scores. Similarly, calf behavior, weight, and sex influenced cow-calf interactions. Both Training and Control groups tended to be protective cows, but the Training group had a higher percentage of calmer cows during the first handling of their calves.

‘Touching’ was a prevalent cow behavior in the post-calving period. Several factors influence postpartum behavior of cows, with calf vigor having an important role in arousing the mother’s interest [34,35]. Male calves were heavier and had more attempts to stand up. Despite being heavier, the higher frequency of attempts combined with the higher latency of males to stand for the first time can be understood as a signal of lower vigor compared to female calves. Lower motility can be a reliable indicator of low vigor in Zebu calves [36]. Indeed, literature reported that male calves have greater chance of poor vigor at birth than females [37,38]. Calf vigor must be considered an important indicator of offspring survival. In Nellore cattle, Schmidek et al. [39] reported risks of low vigor and death were ~ 20% greater in males compared to females.

In our study, heavier calves also experienced a delay in being touched by their dams. One possible explanation was exhaustion from the calving process. Although dystocia did not occur in our study, cows delivering heavier calves can experience severe pain or exhaustion during calving [40]. This phenomenon was evinced by correlations between cows and calf behaviors (longer latency to stand up was related to less maternal touch and interactions). Edwards and Broom [34] reported the influence of weariness on delayed standing after calving, which can also affect the first maternal care (e.g., touching, cleaning and suckling). Additionally, pain signs and weariness can be more evident in primiparous than multiparous cows [30,41], and our studied cows were all primiparous. Future studies on this topic could include calving duration as a possible indicator that would allow identifying cows that had more strenuous effort to push heavier calves, resulting in longer periods of rest to recover before approaching the calf.

In addition to vigor, calf sex appears to have an important role in triggering cows’ nursing behavior. Female calves were touched and interacted more with their dams than males during post-calving period. This behavior can be attributed to the exhaustion from the calving process. As discussed above, male calves were heavier, what might result in a more exhausting calving process. The detrimental effects of a difficult calving process as pain and fatigue have already been reported and may result in im-paired maternal care [34,40,42]. Stěhulová et al. [43] studying Gasconne cattle reported that cows provided more maternal care to the low-weight calves, and licked and followed more female than male calves. Similarly, to taurine cattle, Zebu cows of Guzerat breed also were described as spending more time and providing more maternal care to low-weight calves [44].

Training influenced maternal care as Training group cows spent less time performing maternal care behaviors (less ‘Touching’ and more ‘Not interacting’) than cows in the Control group. Apparently, no previous studies evaluated effects of a training protocol to the first milking and/or use of gentle tactile stimulation on maternal behavior in Zebu cattle. However, some studies have evaluated its effects on behaviors of other cows. Reduction of fear, reactivity, and aversiveness during the first milking in response to gentle tactile stimulation has been reported for Gyr and Holstein-Gyr cows [13-15]. One possible explanation for the lower maternal care of Training group could be related to the higher frequency of cows with lower scores for dis-placement (considered calmer cows) and lower neophobia in these animals. The presence of offspring can cause anxiety and neophobia in primiparous calved females [35]. However, once training protocols can reduce the neophobic response to the environment (including humans and facilities, for example), those cattle may be calmer and more relaxed, resulting in fewer maternal interactions. Indeed, less reactive and calmer dams have been previously reported with less intense maternal care [45,46]. Another interesting hypothesis that may explain, in part, this phenomenon may be related to redirection of maternal care. Mandel and Nicol [47] suggested that dams may redirect their maternal behavior toward sources that provide the tactile stimulus that masks contact with the calf. Although it would be a valid expectation, we do not have data to support this hypothesis. We can only assume that gentle handling and training protocols may modulate some aspects of maternal behavior towards their calves.

Regarding maternal protectiveness, Control group cows tended to be more protective and moved more during the first handling of their calves, probably expressing more agitation. Habituation and gentle interactions decrease cattle aversive and avoidance reactions towards people, and improve human-animal interactions [10,11,48]. This effect appears to remain even in the maternal context. Evaluating beef Gyr, Brahman, and Gyr-Brahman cross cows, Orihuela et al. [23] observed that more aggressive cows towards handlers were those with more intensive protection behavior reaction to separation from their calves during handling. However, the authors did not report any relationship between aggressive behaviors and cow temperament in the peripartum period [23]. Beef cows are recurrently described to be more protective of their calves than dairy cows [27,49]. Using qualitative behavior assessment, Ceballos et al. [31] reported that dairy Holstein-Gyr cows with aggressive behavior during their calves handling were those considered more ‘frightened’ and ‘irritated’ than cows classified as ‘loving’ and ‘attentive’. Although, in general, Zebu dams demonstrate strong protective behavior to their calves, we emphasize the plasticity of this phenotype, influenced by several factors [25,50]. In this case, environmental effects and handling routine may modulate maternal behavior. Repeated handling and habituation are useful tools to improve animal responses to handling [50,51]. Cows with lower milking reactivity, better productivity, and calmer response when restrained were al-ready described as a result of positive tactile stimulation protocol [13,14]. We also attribute the less intensive maternal protective response to the training protocol. Cattle habituated to human presence and gentle handling may influence how cattle perceive humans [23]; they may regard humans as something other than a potential risk for their calves. Thus, in addition to the already reported productive and profitable benefits, Gyr dairy cows submitted to gentle tactile stimulation also appeared to be more calm and relaxed toward the handling of their calves.

Our primary hypothesis was that the training protocol would affect the cows’ maternal behavior. Indeed, our results supported this hypothesis. Control group cows spent more time performing nursing behavior and touching their calves. Our second hypothesis was that Training group cows have lower protective response against handlers during the first handling of their calves. Our results also supported this hypothesis, demonstrating that the training protocol for the first milking involving gentle tac-tile stimulation decreased protective responses. Training group cows tended be less protective and moved less during the first handling of their calves. In general, our findings bring new perspectives about the use of training protocol to habituate primiparous cows to the first milking. In addition to the relevant benefits already known on productivity and animal welfare, adoption of a training protocol could reduce aggressive responses of dams to management of their calves. In a dairy farm context, the absence of strong maternal care in Zebu females could help to promote the intensification of milking procedures as in taurine breeds, mainly Holsteins, that have been milked without their calves for decades [19,46,50].

Although the effects documented herein suggest a causal relationship between training protocol and maternal performance, our study had some limitations, requiring thoughtfulness to extrapolate our results. Even though we observed a tendency of the training protocol to affect maternal protectiveness (MCS score) of Gyr dairy cows, differences between individuals were important. Some aspects of these individual traits may influence stimuli response even in standardized protocols [15]. Individuals' past experiences (before conception or even during gestation) may have masked or influenced the behavior of the cows in some way, mainly toward the handlers [23,48]. Furthermore, we only had 16 primiparous cows in the Training group and a single group per treatment (without replication). Therefore, the pre-milking training effects on maternal protectiveness must be confirmed in future studies with greater sample sizes and preferably using a replicated design with both male and female offspring. Moreover, it is important to evaluate intra-observer reliability when measuring animal behavior as it gives confidence about the quality of assessment made by the same observer, unfortunately, we did not evaluate it in our study; nevertheless, the observer has been trained in observing animal behavior with years of experience in this task. Future studies should consider including a baseline (pre-calving) reactivity assessment of both groups (trained and control) to better elucidate the possible effects of the training protocol on cows' reactivity post-calving. Finally, more studies should investigate possible effects of training protocols for longer intervals after calving, evaluating long-term effects on cow-calf bonds across lactation in cow-calf contact production systems.

5 CONCLUSIONS

A pre-calving training protocol for the first milking involving gentle tactile stimulation on primiparous Gyr dairy cows was associated with calmer and relaxed behavior on the first handling of their newborns, which was beneficial for the handling routine of calves. There was more safety for handlers, with potential to improve welfare of animals and humans involved in the calf first handling. However, primiparous Gyr dairy cows subjected to the training protocol spent less time touching their calves. In addition, maternal care was also affected by calf vigor, weight, and sex. Potential long-term effects of lower maternal care by trained cows on calf development need to be determined.

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6 FINAL CONSIDERATIONS

The increase in livestock efficiency is based on several factors, among which the herd reproductive efficiency and adequate human-animal relationships have significant importance. The livestock research advances has allowed the modernization of processes and widespread the use of technological devices and animal data applied to production purposes. Currently, most studies are related to European cattle raised in temperate and/or artificial conditions. In the tropical regions, there is still a lack of studies about the impact of management and applied technologies in dairy herds composed mainly by Zebu animals. Since Zebu breeds have several particularities related to behavior and physiology, studies related to the use of applied technologies and new management strategies might help in the productive and economic development of tropical herds. Thus, this thesis brings important and hitherto unpublished information about the physiology and behavior as well as the effect of handling protocols applied to a dairy Zebu breed in the reproductive context.

For the first time in the scientific literature, intra-ruminal devices revealed that Gyr nulliparous heifers showed changes in activity and reticulo-rumen temperature consistent with approaching parturition. Like European cattle, Zebu heifers showed the same pattern of changes in both traits, reflected in increased activity and decreased temperature. The increase in activity was most evident in the 11 hours prior to calving, preceded by a drop in temperature at 21 hours before calving. Interestingly, the drop in reticulo-rumen temperature was 0.20 °C, a magnitude lower than that recorded for European cattle. These findings provide important practical information, indicating that activity and reticulo-rumen temperature can be indicators of calving in nulliparous Gyr heifers. Additionally, differences in thermal physiology between Zebu and European cattle should be taken into consideration when reticulo-ruminal temperature is used for calving prediction in devices validated for European cattle only. In the future, calving prediction based on intraruminal devices may assist farmers that used Zebu breeds under tropical conditions as have been made with European cattle, enabling earlier and more efficient calving assistance.

As important as the calving process itself, the cows' behaviors in the peripartum must be considered as indicators of health conditions and maternal care. Our results showed that parity is an important factor that influences the behavior of cows in the peripartum period. Primiparous cows showed more signs of pain and discomfort than multiparous cows during parturition, which directly influenced the cow-calf interactions. In a practical context, these results reinforce the need for frequent inspections of primiparous cows in the peripartum, suggesting better chances of guaranteed assistance in cases of exhaustion and obstetric problems. Regarding maternal behavior, the Gyr cows could be labeled as 'Protective-aggressive mothers' and 'Protective-attentive mothers', which 'Protective-attentive mothers' being better mothers and presenting a lower risk of danger to the handlers. Both parity groups were protective but only multiparous were aggressive toward the first handling of their calves. Aspects related to maternal care and defense are important to ensure better chances of offspring survival. However, the aggressive conduct of cows may be a problem for caretakers' safety. Based on our findings, we suggest that during the first handling of the calves, more caution should be considered when handlers face multiparous cows, as there is a greater chance of presenting aggressive behavior.

In addition to the cows' behavior, other factors influenced the maternal conduct, such as environmental stimuli and offspring traits. For primiparous Gyr cows, our results demonstrated that the calf's behavior, weight, and sex played important role in modulating the maternal care. Female calves were those who presented less weight but better vitality, traits that may have triggered more intense maternal care by the cows. In addition, the training protocol to the first milking involving tactile stimulation also influenced the maternal care. Cows submitted to the protocol were those with reduced defensive behavior but also spent less time touching their calves. This relationship may have been result of the reduction of neophobic response to novel stimuli, attributed to habituation to the training protocol. This finding argues that, in addition to the benefits of productive and profitable aspects, the use of training protocol to the first milking involving tactile stimulation can reduce the extreme aggressive response and strong maternal bond in Zebu cows. Therefore, the adoption of this kind of protocol can improve the handling in both caretakers' security and farm productivity contexts.

In general terms, this thesis brings new perspectives for the adoption of technologies and management protocols applied to Zebu animals raised in tropical conditions. We confirmed previous studies that validated these types of tools in European cattle, but they had not yet been studied in Zebu cattle. These methodologies, whether technological devices or handling improved, can be also used for Zebu breeds, but some particularities of behavior and physiology must be considered. More studies that evaluate the use of these new technologies and handling resources in Zebu breeds will contribute to the advances of the tropical livestock production chain.

APPENDIX OF THESIS

APPENDIX A - Predictive potential of activity and reticulo-rumen temperature variation for calving in Gyr heifers (*Bos taurus indicus*) published on Journal of Thermal Biology (2021). Source: https://doi.org/10.1016/j.jtherbio.2020.102793

APPENDIX B - Is maternal defensiveness of Gyr cows (*Bos taurus indicus*) related to parity and cows' behaviors during the peripartum period? published on PLos One (2022). Source: https://doi.org/10.1371/journal.pone.0274392

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Predictive potential of activity and reticulo-rumen temperature variation for calving in Gyr heifers (*Bos taurus indicus*)

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ABSTRACT

Factors related to the thermal physiology and activity of Zebu animals close to calving are still unknown. The aims of this study were 1) to describe the pattern of reticulo-rumen temperature and activity variation in nulliparous Gyr heifers close to calving, and 2) to evaluate the predictive potential of these traits for calving in Gyr heifers. Forty pregnant Gyr heifers that had calved between August and December 2017 at the Getúlio Vargas Experimental Station, Empresa de Pesquisa Agropecuária de Minas Gerais (Epamig), Brazil, were used. The animals received a rumen bolus to monitor reticulo-rumen temperature (Trr) and activity (Act) at intervals of 10 min. Mixed linear models were used. A decrease in Trr and an increase in Act were observed on the days preceding calving. Differences in Trr and Act were more evident during the final 21 and 11 h previous to calving compared to 14 days before calving, measured at the same time of day. There was a decrease of about 0.20 °C in Trr at the time of calving when compared to baseline (14 days before calving measured at the same time of day). Environmental variables, such as temperature and air humidity, as well as biological factors such as circadian rhythm, may influence the interpretation of the data. In conclusion, variations exist in the Trr and Act of Gyr heifers in the hours before calving, which is preceded by a decrease in Trr and an increase in Act. Particularities in the thermal physiology of Zebu cattle must be considered when prediction devices previously validated only for European breeds are used.

1. Introduction

The productive performance of cattle is directly related to reproductive success, which is determinant for the lactation of cows. Calving disorders can have genetic and non-genetic causes and can compromise the survival of the cow and calf (McGuirk et al., 2007). Monitoring animals during labor helps identify these possible problems and enables to provide obstetric assistance when necessary. However, the in-person inspection of the calving process is time consuming and labor intensive. Therefore, animal monitoring technologies such as temperature sensors and sensors recording behaviors such as feed intake, rumination and activity have been gaining space on farms, assisting with their productive management by predicting events such as estrus (Cooper--Prado et al., 2011; Dolecheck et al., 2015) and calving (Costa Jr. et al., 2016; Borchers et al., 2017; Ricci et al., 2018; Keceli et al., 2020). Precision techniques that assist in the automated detection of events related to animal reproduction, mainly the calving, contribute to herd management since they enable the accurate and reliable real-time prediction of key events that require management actions.

Changes in the movement and activity of the animal can help predict calving (Borchers et al., 2017). Huzzey et al. (2005) reported a tendency towards longer standing times of Holstein cows during the prepartum period. In the hours before calving, an increase in activity and movement is observed, as well as a decrease in water and feed intake

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RESEARCH ARTICLE

Is maternal defensiveness of Gyr cows *(Bos taurus indicus)* related to parity and cows' behaviors during the peripartum period?

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Abstract

The maternal care of cows can influence both the milk production and the performance of their calves, making this a topic of important relevance for the production industry that uses zebu cattle. The aims of this study were to 1) investigate the effects of parity on the behaviors of Gyr cows during the peripartum period; 2) characterize the maternal defensiveness of primiparous and multiparous cows towards handlers during the first handling of their calves; and 3) evaluate the relationships between cows' behaviors at the peripartum period and maternal defensiveness. Thirty-one Gyr cows (primiparous and multiparous), from Empresa de Pesquisa Agropecuária de Minas Gerais (Brazil), were used. The animals were placed in a maternity paddock monitored by video cameras. The behaviors of the animals were collected in four periods: Pre-calving, Post-calving, First handling of calf and Post-handling. Primiparous cows presented more pain signs, reflected in arched spine (P = 0.05), and tended to move more (P = 0.07) than the multiparous in the Pre-calving period. Trends were observed for both Maternal Composite Score (P = 0.06) and Maternal Protective Behavior score (P = 0.06), indicating that both primiparous and multiparous were protective, but only multiparous cows were aggressive toward the caretakers on the first handling of their calves. The most protective cows spent more time eating during the prepartum period (P = 0.03), while the least attentive cows spent more time lying down (P = 0.02) in the prepartum period. The cows who nursed and stimulated their calves more were also calmer (P = 0.02) and more attentive (P = 0.01). In conclusion, the peripartum behaviors of Gyr cows were related to maternal care and maternal defensiveness. Multiparous cows tended to be more aggressive than primiparous cows at the time of the first handling of their calves.

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