

JOÃO PAULO FERREIRA GOMES

**FREESTALL BEDDING MATERIALS FOR DAIRY COWS AND
COLOSTRUM REPLACER FOR BEEF CALVES**

Dissertation submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

Adviser: Polyana Pizzi Rotta

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“Faça o teu melhor, na condição que você tem, enquanto você não tem condições melhores, para fazer melhor ainda!”.

(Mário Sérgio Cortella)

ABSTRACT

GOMES, João Paulo Ferreira, M.Sc., Universidade Federal de Viçosa, December, 2022. **Freestall bedding materials for dairy cows and colostrum replacer for beef calves**. Adviser: Polyana Pizzi Rotta. Co-adviser: Alex Lopes da Silva.

The aim of the first study was to evaluate sand or sawdust materials for freestall system. Sixteen Holstein cows were used in this trial, with an average production of 38 ± 9.0 L/d. Eight cows were allocated in sand beddings and 8 cows were allocated in sawdust beddings, receiving three dailies' meals, and milked three times a day. The experimental period lasted 30 d, preceded by a 7 d adaptation period. Animal behaviour, milk yield, somatic cell count, and the hygiene score of the thigh, legs, and udder were evaluated. Samples of sand and sawdust were collected for microbiological evaluation and the surface temperature of the beds was also measured. The type of bed did not affect milk production and the lying behaviour of the cows. The sawdust bed had a greater count of enterobacteria. For staphylococcus count, sand had a greater value at d2; however, for sawdust at d28 the staphylococcus count was greater than in sand beddings. The cows kept in the sawdust bed showed greater somatic cell count, but no case of clinical mastitis for both materials was observed. The sand bed warmed up more than the sawdust, but there was a loss of temperature more quickly at night. The results obtained in this study demonstrate that sawdust may be used to replace sand, but care with handling bed replacement must be considered to ensure the quality of the material for longer periods. The second study aimed to evaluate the influence of colostrum replacer on health and performance of beef calves. Two experiments were carried out. In the first one, 206 $\frac{3}{4}$ Angus \times Nellore calves, offspring of $\frac{1}{2}$ Angus \times Nellore primiparous cows were used. Calves were divided into 3 groups: NRG (no replacer group) (n = 68), 50I (50 g of IgG supply) (n = 68), and 100I (100 g of IgG supply) (n = 70). The serum protein level was assessed using three methodologies: optical brix refractometer, digital brix refractometer, and serum total protein refractometer (STP-REF). In the second, 247 Nellore calves, offspring of Nellore cows were used. Calves were divided into three groups: NRG (n = 85), 50I (n = 81) and 100I (n = 81). The serum protein level was assessed as the experiment 1. The animals were weighed using weighing tapes at calving and weaning at 80 days of age. For the first experiment, there was no difference ($P > 0,05$) for colostrum efficiency regardless of the method

used and gender. In the second experiment, an effect was observed ($P = 0.02$) on STP concentrations measured by the STP-REF and through the digital brix refractometer in the 50l group. Serum total protein evaluated through the optical brix refractometer did not vary among treatments as well as ADG. The effect of colostrum replacer was different from Nellore pure calves and $\frac{3}{4}$ Angus \times Nellore calves, with greater passive transfer efficiency for Nellore calves. However, for dams well fed like in these experiments, no effect on morbidity, mortality and animal performance were observed for calves.

Keywords: Animal welfare. Immunoglobulin G. Milk quality. Passive Immunity. Sand. Sawdust.

RESUMO

GOMES, João Paulo Ferreira, M.Sc., Universidade Federal de Viçosa, dezembro de 2022. **Materiais de cama freestall para vacas leiteiras e substituto de colostro para bezerros de corte.** Orientadora: Polyana Pizzi Rotta. Coorientador: Alex Lopes da Silva.

O objetivo do primeiro estudo foi avaliar materiais como areia ou serragem para sistema freestall. Dezesesseis vacas holandesas foram utilizadas neste ensaio, com produção média de $38 \pm 9,0$ L/d. Oito vacas foram alocadas em camas de areia e 8 em camas de serragem, recebendo três refeições diárias e ordenhadas três vezes ao dia. O período experimental durou 30 dias, precedido por um período de adaptação de 7 dias. Foram avaliados o comportamento animal, produção de leite, contagem de células somáticas e o escore de higiene da coxa, pernas e úbere. Foram coletadas amostras de areia e serragem para avaliação microbiológica e também medida a temperatura superficial dos leitões. O leito de serragem apresentou maior contagem de enterobactérias. Para contagem de estafilococos, a areia teve maior valor no d2; entretanto, para serragem no d28 a contagem de estafilococos foi maior do que em leitões de areia. As vacas mantidas em cama de serragem apresentaram maior contagem de células somáticas, porém não foi observado nenhum caso de mastite clínica para ambos os materiais. O leito de areia aqueceu mais que a serragem, mas houve perda de temperatura mais rapidamente à noite. Os resultados obtidos neste estudo demonstram que a serragem pode ser utilizada em substituição à areia, mas cuidados no manuseio da substituição do leito devem ser considerados para garantir a qualidade do material por períodos mais longos. O segundo estudo teve como objetivo avaliar a influência do substituto do colostro na saúde e desempenho de bezerros de corte. Dois experimentos foram realizados. No primeiro foram utilizados 206 bezerros $\frac{3}{4}$ Angus \times Nelore, descendentes de vacas primíparas $\frac{1}{2}$ Angus \times Nelore. Os bezerros foram divididos em 3 grupos: NRG (grupo sem substituto) (n = 68), 50I (50 g de suprimento de IgG) (n = 68) e 100I (100 g de suprimento de IgG) (n = 70). A proteína sérica O nível de proteína foi avaliado por meio de três metodologias: refratômetro brix óptico, refratômetro brix digital e refratômetro sérico de proteína total (STP-REF). No segundo, foram utilizados 247 bezerros Nelore, descendentes de vacas Nelore. Os bezerros foram divididos em três grupos: NRG (n = 85), 50I (n = 81) e 100I (n = 81). O nível de proteína sérica foi

avaliado conforme experimento 1. Os animais foram pesados com fitas de pesagem no parto e no desmame aos 80 dias de idade. experimento, não houve diferença ($P > 0,05$) para eficiência do colostro independentemente do método utilizado e sexo. No segundo experimento foi observado efeito ($P = 0,02$) nas concentrações de STP medidas pelo STP-REF e através do refratômetro digital brix no grupo 50 I. A proteína sérica total avaliada através do refratômetro óptico brix não variou entre os tratamentos, assim como o GMD. O efeito do substituto de colostro foi diferente para bezerros Nelore puro e bezerros $\frac{3}{4}$ Angus \times Nelore, com maior eficiência de transferência passiva para bezerros Nelore. Entretanto, para mães bem alimentadas como nestes experimentos, nenhum efeito sobre a morbidade, mortalidade e desempenho animal foi observado para bezerros.

Palavras-chave: Areia. Bem-estar animal. Imunoglobulina G. Qualidade do Leite. Serragem.

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CHAPTER 1 - FREESTALL BEDDING MATERIALS FOR DAIRY COWS

1.1. INTRODUCTION

Despite the high investment, there are several advantages associated with the freestall system, which in addition to the economic benefits, are highlighted in improving the quality of life and consequently increasing the level of milk productivity (Wolfart, 2022).

The choice of the type of material to be used as bed for cows may influence MY, health, and welfare (Santos, 2015). Bedding material also influences the cow's hygiene score and, consequently, the quality of the milk. Organic bedding should be managed more intensively than inorganic bedding and has been attractive due to the possibility of later use as organic fertilizer (Leso et al., 2020). Organic materials such as sawdust and wood shavings tend to show greater growth of microorganisms than inorganic materials (sand), as the material presents a substrate for the growth of bacteria (Santos and Freu, 2018). Zdanowicz et al. (2004) reported that cows housed in sawdust bed had a greater coliform and *Klebsiella* spp. concentrations in teats; however, cows housed in sand beds had a greater concentration of *Streptococcus* spp. at the edge of the ceilings. Therefore, bedding material must have low microbial growth, adequate moisture drainage, and easy cleaning (Dibbern and Santos, 2013) to avoid pathogens proliferation which may cause mastitis and other disorders (Gantner et al., 2017).

Another important point to be highlighted is the treatment and destination of waste in dairy farms. In freestall systems, the entire volume of excrement is removed by scraping or washing (flushing), generating a large volume of waste (Caldato et al., 2019). When using sand, it is necessary to separate it from other waste to prevent its deposition in tanks or passage boxes, in addition of preventing the clogging of pumps. When adding sand to the crop, the soil becomes sandy; Carneiro et al. (2019) observed that in sandy soils, iron, and aluminum, even in small amounts, influence the leaching of phosphorus. However, for sawdust, as it is an organic material, it will be mixed with the waste in the washing and may be used as a fertilizer and in those cases where the installation is scraped, the sawdust together with the solid waste can be composted and used as an organic fertilizer, making its use an interesting

alternative. Finally, there is no study in the literature comparing the use of sand and sawdust in freestall systems in tropical climate.

In this sense, the objective of this study was to compare sand or sawdust for freestall system on animal behavior, MY, sand and sawdust bed microbial populations, and animal health. The hypothesis is that sawdust will be able to replace sand, ensuring good MY, good hygiene, and animal well-being.

1.2. MATERIALS AND METHODS

The experiment was conducted at Animal Science Department at Universidade Federal de Viçosa (Viçosa, MG, Brazil) following the standard procedures for animal care and handling according to the university's guidelines (109/2022). The trial was conducted during the spring. According to Köppen's classification, the region's climate was Cwa type, with two well-defined seasons, with hot humid summers and cold dry winters. The average annual rainfall was 1229 mm and the average temperature was 20.6 °C.

1.2.1. Treatments and Design

The experiment lasted 60 d, with 21 d for adaptation of the cows to environmental conditions and 7 d for adaptation to bed. The study was carried out in a freestall system with 16 lactating Holstein cows, with an average production of 38 ± 9.0 L/d, BW of 600 ± 59.0 kg, DIM of 125 ± 91.0 d and average lactation order of 2.2 ± 1.10 . The animals received three meals a day (0630, 1200 and 1430h) consisting of corn silage, Tifton hay, cottonseed whole, soybean meal, ground corn meal, mineral and vitamin premix with free access to water.

Cows were divided into two groups of 8 animals each. Each group received a type of bedding material (sand (a) or sawdust (b) (Fig. 1)). Each pen had 12 individual beds, but 4 beds were isolated (Fig. 2) in each pen so bed:cow ratio was 1:1, therefore, 16 single beds were used for the two groups of 8 animals. Bed management consisted of removing fecal plates twice a day, followed by spreading the material to tidy the bed surface and once a week the material was added.

1.2.2. Bed Temperature

The HOBO data logger (model U23 Pro v2, Onset Computer Corp., Bourne, MA) (precision of $\pm 0.25^{\circ}\text{C}$ from -40 to 0°C and $\pm 0.2^{\circ}\text{C}$ from 0 to 70°C for temperature and $\pm 2.5\%$ accuracy when RH is 10 to 90% and $\pm 5\%$ when RH is below 10% and above 90%) was installed in the freestall system at 1.10 m height (to estimate temperature (**T**) and relative humidity (**RH**)). Thermal environment data were collected during all days of the trial at intervals of 10 min. With the thermal environment data, the Temperature and Humidity Index (**THI**) was calculated according to the equation developed by Zähler et al. (2004): $\text{THI} = 0.8 \times T + \text{RH}/100 \times (T - 14.4) + 46$. The data for T, RH, and THI over the days are presented in Figs. 3; 4.

Bed temperature (sand and sawdust) was measured at d10, d17, d24, and d31 (Figs. 3; 4). The temperature collections were performed with an interval of 2 h, totaling 12 results for each day. The temperature was measured with a calibrated digital skewer thermometer (TermoMed, Incoterm model 6132, C. Technology Ltd., Porto Alegre, Brazil) with an accuracy of $\pm 1^{\circ}\text{C}$ (-20°C to $+80^{\circ}\text{C}$) and $\pm 2^{\circ}\text{C}$ (-45°C to -19.9°C and of 80.1°C to $+200^{\circ}\text{C}$).

1.2.3. Milk Yield and Quality

Cows were milked three times a day: 0630, 1430, and 2030h, and during the study, MY was recorded once a week, totaling four collection periods. Individual milk samples were also collected to perform SCC via the Somaticell® (Madasa, Sao Paulo, Brazil) rapid test once a week, totaling four analyses. According to Rodrigues et al. (2009), the on-farm Somaticell® rapid test showed adequate ability to determine CCS in milk samples.

1.2.4. Animal Behavior

For behavioral assessment, the experiment was divided into 3 periods: 1 (d19 and d20), 2 (d26 and d27) and 3 (d33 and d34). Thus, the observation of animal behavior was carried out at d19, d20, d26, d27, d33, and d34, totaling 48 h of observation per period. Animal behavior was evaluated using the visual measurement method with observation intervals every 5 min. The following behaviors

were recorded in a spreadsheet: 1) standing up ruminating, 2) standing up resting, 3) feeding, 4) drinking water, 5) lying down ruminating, and 6) lying down in idleness. The evaluation of the events was done following the methods described by Zambelis et al. (2019).

The methodology described by DeVries et al. (2003) was used to estimate the meal criteria, mealtime, activity frequency (events/d) and activity duration (mean duration/event) for rumination, feeding, lying, and standing behaviors. Briefly, intervals between events were calculated and transformed into log₁₀ using Excel spreadsheets. Meal criteria was determined per animal per period, as the point at which the intrameal interval distribution curve is crossed by the intermeal interval distribution curve (Figs. 5; 6). Meal criteria data were used to calculate the number of events (events/d), counting the interval numbers that exceeded the criterion and adding 1 (Zambelis et al., 2019). To calculate the average duration per event (min/event) the onset times were considered, from the first session of the event to the end of the last session of the event. The total time of each event was calculated by the sum of the two activity durations described above.

1.2.5. Bedding Microbiology and Cleaning Score

Beds (sand or sawdust) were replenished once a week. Composite samples from bed 1 to 4 and 5 to 8 of each pen (Fig. 2) were made once a week, stored in falcon tubes for microbiological analysis. Microbiological analyses were performed using the microdrop plating method where 1 g of composite sample, representative of the stalls, was homogenized (1:10) in peptone solution, successively diluted (100 to 10¹⁰) and plated on selective Macconkey agar medium (Kasvi, Spain) for enterobacteria and Salt mannitol agar (Kasvi, Spain) for staphylococci. The microdrop plating technique was used, using an aliquot of 0.02 mL with three replicates per sample, as described by Morton et al. (2001).

$$\text{CFU/mL} = \text{average number of colonies} \times \text{DF}$$

Where:

CFU/mL: colony forming units per mL of the original sample;

DF: dilution factor (inverse of the dilution where between 8 and 80 colonies were found).

The cleaning score was evaluated in 3 locations in the animal (udder, thigh, and legs) and assigned grades ranging from 1 to 4, as follows: clean (1), slightly dirty (2), dirty (3), and very dirty (4). Pictures of the animals were taken at d8, d15, d22, and d29 and the images were randomly evaluated, so the score analysis could be performed minimizing the effect of bed and week. To assist in the classification of images, a score card (Fig. 7) developed by Dohmen et al. (2010) was used. Points were assigned for the right thigh, left thigh, right leg, left leg, and udder of each animal and the thigh and leg scores were averaged to generate the left + right thigh and left + right leg scores.

1.2.6. Statistical Analyses

Statistical analysis of production, SCC, and cleanliness scores were performed using the SAS Software (SAS, University Edition) considering a completely randomized design in a repeated-measures-in-time scheme. Behavior analysis, microbiology, and temperature were carried out considering a completely randomized design using the Mixed Proc of the SAS Software (SAS University Edition). Statistically significant variables in analyses were considered when $P < 0.05$. And the variables with p value between 0.05 and 0.10 were considered as a trend.

1.3. RESULTS AND DISCUSSION

1.3.1. Bed Temperature

The average temperature of the sand and sawdust beds increased over the days. The sawdust and sand beds differed ($P < 0.05$) over all days of evaluation; however, sand had a greater average daily temperature in 1st and 2nd day of evaluation, but after the second day, sawdust had a greater average daily temperature. The temperature measurement of the beds was carried out 3 d after beds renewal and over the days, the temperature in freestall system underwent some fluctuations, but in general the ambient temperature increased and this could influence the temperature of the beds.

Specific heat is a characteristic of the material that determines the amount of energy needed for each gram of material to undergo a variation of 1°C (Oliveira et al., 2022). As of d17, both materials ended up heating up more than at the beginning. However, the sand loses temperature faster than sawdust, with lower average temperatures while sawdust takes a little longer to heat, but it stays hotter for a longer time, which generates a higher average temperature over the days when compared to sand (Fig. 8).

From mid to late afternoon (1600 to 1800h), the mean temperature of the sand bed was greater than that of the sawdust bed ($P < 0.05$). In the early morning period, the opposite was observed, the sawdust bed had a greater temperature ($P < 0.05$). Cecchin (2014) observed that the behavior of lying down in the stall may be affected by the capacity of the material used to absorb or dissipate heat. Wagner-Storch et al. (2003) evaluating rubber mattresses, water mattresses, rubber mats, concrete, and sand, stated that the occupation of the beds decreased as the temperature increased. Cows preferred sand at medium temperatures (-6 to 15 °C), but at lower temperatures (-17 to -6 °C) cows preferred a waterbed compared to a rubber and concrete mat, but at greater temperatures (27 to 38 °C) the occupancy rate of the beds reduced, suggesting that the ambient temperature, as well as the temperature of the pens may affect the choice of the animal for a certain type of material.

Sand has a better thermal conductivity compared to sawdust, that is, it will facilitate the heat exchange process between the animal and the bed, ensuring better thermal comfort for the cow, especially in situations of high temperatures (Fig. 9). However, in the present study, the type of bed did not affect the lying behavior of the animals, suggesting that the bed temperature did not cause discomfort for the animals in this trial.

1.3.2. Milk Yield and Quality

The type of bedding evaluated in this study did not influence ($P = 0.43$) MY (Table 1), probably due to the good conditions maintained for the different bed surfaces. There is no study comparing sand and sawdust beds in tropical climate. However, Andreasen and Forkman (2012) evaluated mattress bed, rubber mat, and sand during fall and winter in Denmark and observed a greater milk production for cows kept in sand bed. Furthermore, Calamari et al. (2009) tested for a period of 50 d, straw mat,

rubber mat, mattress, and sand in bed and observed a greater milk production for cows housed in sand bed. Sawdust bed may be an interesting alternative in freestall system as it did not affect MY of high-yielding cows as observed in this study. However, it is important to emphasize that this study was carried out for a short period and longer studies would be interesting to understand how sawdust behaves over time.

The mean SCC (Table 1) was greater ($P = 0.04$) when kept in sawdust bed compared to sandbed. Although the cows housed in sawdust bed had an average of 8.7% greater SCC, the results obtained in this study showed that the evaluated system had good bed management, in addition of being efficient in controlling clinical mastitis (no cases during the experiment was observed). Sawdust, as an organic material, may present a greater development of microorganisms and because it has very fine particles, it tends to adhere more easily to teats, increasing the risk of intramammary infection (Dibbern and Santos, 2013). Zdanowicz et al. (2004) found a moderate correlation between the bacterial count at the tip of the cow's teats and the bacterial count in the sawdust bed and a weak relationship for the sand beds, suggesting that the physicochemical characteristics of the sand favor a lower contamination in temperate climate region. By favoring an adequate and animal-friendly environment, it will influence the cow's hygiene, reducing the incidence of mastitis and improving milk quality and production (Leso et al., 2020).

In a study conducted by Oliveira et al. (2020), with Holstein animals in freestall under tropical conditions, it was concluded that eucalyptus wood shavings and ground corn cob are efficient to be used as organic bedding, as they resulted in low SCC, and did not interfere in cases of mastitis.

1.3.3. Animal Behavior

The type of bed did not influence ($P = 0.22$) the total time in minutes of standing, standing added to the small intervals that animals were lying down (Meal time), and the time of the small intervals that animals were lying down (Meal criteria). For the variable referring to the number of times the animal stood up (events/d), there was a tendency ($P = 0.08$) for the animals kept in sand beds to get up more times a day (about 14.7 %) when compared to animals kept in sawdust bedding. The type of bed did not influence ($P = 0.21$) the mean duration of mealtime, that is, there was no bed

influence for the duration that the animals were standing in each event containing the intervals in which the animals lay down and if they got up again.

The type of bedding did not influence ($P = 0.18$) the total time that animals were lying down, the Meal time ($P = 0.15$), the Meal criteria ($P = 0.93$), the number of times the animals lay down (events/d) ($P = 0.75$). Tucker et al. (2003) tested the effect of different bedding on the preference of dairy cows and observed that the animals preferred sand and sawdust bedding instead of a geotextile mattress bed covered with 2 to 3 cm of sawdust. It was also observed that cows lay down more and spent more time lying on sand and sawdust beds and spent more time standing when restricted to stalls with mattresses.

When evaluating the behavior of cows on different surfaces, Calamari et al. (2009) found that cows spent more time lying on sand and straw than on mattresses and rubber mats, in addition, it was observed that the number of events/d was greater on sand and straw, but there was no difference in duration average/event and the mattress treatment presented longer standing time.

These results show that the type of bed may affect the animal's preference and that cows prefer soft surfaces such as sand or straw. However, soft beds provide more comfort and improve animal welfare (Kull et al., 2017). Sawdust is an organic material that supports the growth of bacteria and has a high carbon ratio; however, the main feature of this material is the ability to mold to the animal body and offer comfort for the animal to lie down. However, in the present study, the lying behavior of the animals was not affected ($P = 0.66$) by the type of material used in the bed, showing that sawdust is also a comfortable material for the animals to lie down on.

In the present study, there was no effect ($P = 0.37$) of the bed in relation to the total feeding time, the Meal time ($P = 0.16$), the Meal criteria ($P = 0.82$), number of times the animal went to the trough and mean duration of Mealtime ($P = 0.51$). For the average time that the animals were fed, we observed a tendency ($P = 0.07$) for the animals kept in the sawdust to spend more time (17.7%) feeding at each meal.

When analyzing the total rumination time of the animals, there was a trend ($P = 0.07$) of greater rumination (about 6.04%) for the animals kept in the sand bed. However, there was an effect ($P < 0.01$) for the Meal time, and the cows kept in sawdust beds had a greater value for this evaluation. Furthermore, there was an effect ($P = 0.01$) for the average duration of Meal time, indicating that the animals spent more time ruminating per event in the sawdust bed compared to Meal time.

The type of bed did not influence ($P = 0.61$) the Meal criteria nor the mean time for each rumination event. Animals kept on the sand bed tended ($P = 0.10$) to have a greater number (12.4%) of rumination events per day, that is, animals on sawdust had fewer rumination events, but each event was longer, on the other hand, in the sand the animals had more rumination events, but with a shorter duration.

1.3.3. Animal Behavior

The type of bed did not influence ($P = 0.22$) the total time in minutes of standing, standing added to the small intervals that animals were lying down (Meal time), and the time of the small intervals that animals were lying down (Meal criteria). For the variable referring to the number of times the animal stood up (events/d), there was a tendency ($P = 0.08$) for the animals kept in sand beds to get up more times a day (about 14.7 %) when compared to animals kept in sawdust bedding. The type of bed did not influence ($P = 0.21$) the mean duration of mealtime, that is, there was no bed influence for the duration that the animals were standing in each event containing the intervals in which the animals lay down and if they got up again.

The type of bedding did not influence ($P = 0.18$) the total time that animals were lying down, the Meal time ($P = 0.15$), the Meal criteria ($P = 0.93$), the number of times the animals lay down (events/d) ($P = 0.75$). Tucker et al. (2003) tested the effect of different bedding on the preference of dairy cows and observed that the animals preferred sand and sawdust bedding instead of a geotextile mattress bed covered with 2 to 3 cm of sawdust. It was also observed that cows lay down more and spent more time lying on sand and sawdust beds and spent more time standing when restricted to stalls with mattresses.

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1.3.4. Bedding Microbiology and Cleaning Score

The sawdust bed had a greater count ($P < 0.01$) of enterobacteria in relation to the sand bed, approximately 49.6% greater. There was also a difference ($P < 0.01$) for count of enterobacteria in the evaluated periods. The third period (d14) had the greatest count, followed by the 4th period (d21) which did not differ from d14 and d28.

Staphylococcus count showed an interaction ($P = 0.01$) effect between bed \times period. There was no effect ($P = 0.45$) for bed types. There was a significant difference ($P \leq 0.05$) for staphylococcus count in the sand bed, with greater *staphylococcus* counts compared to sawdust at d21. At d28 the sawdust bed, had a greater count of staphylococcus than sand, with a significant difference between the beds.

When comparing sawdust with sand, it was expected that the organic material will have a greater bacterial count, however, the degree of contamination of the beds increased ($P = 0.001$) over time, this may be due to the fact that the beds are replaced only once a week, allowing accumulation of organic substrate in the materials over the days and favoring the growth of microorganisms in both materials. To minimize such results, it is necessary to increase the periods of bed renewal and reduce the accumulation of substrates for bacterial growth. According to Hogan et al. (1989), organic materials (straw or sawdust) have greater moisture and gram-negative bacteria, coliforms, *klebsiella* and *Streptococcus* counts than inorganic materials (sand, crushed limestone). Zdanowicz et al. (2004) observed that cows housed in sawdust had greater counts of coliforms and *klebsiella* at the tip of the teats and cows housed in sand had greater counts of *Streptococcus* spp. In addition, both sand and sawdust suffered a weekly increase in the count of coliforms, *klebsiella* and *Streptococcus*.

The animals kept in bed with sand had a greater ($P = 0.01$) hygiene score for the left thigh and the average of the left + right thigh ($P = 0.04$; Table 2). Thus, in two points the animals kept in sand were dirtier than cows in sawdust bed. The cleaning score was evaluated using a scale from 1 to 4 and the average for the left thigh was 2.62 for the cows housed in the sand and 2.00 for the animals housed in sawdust beds. For right + left thigh the value was 2.56 and 1.98 for sand and sawdust, respectively, so the thighs are classified as slightly dirty. Therefore, even though the sand had a worse result for the hygiene score, all animals are considered clean. Thus, we may infer that both beds were good to ensure cleanliness and hygiene in animals. De Palo et al. (2006) tested different bedding materials for cows in freestall system and reported that cows preferred Ethylene Vinyl Acetate (rubberized, flexible, and waterproof polymer) over dry manure and wood chips, but under heat stress conditions, with THI > 80, the animals chose to use organic materials (dry manure and wood shavings), which offered greater thermal comfort. Studies showed that sand bed is more hygienic than rubber bed (Andreasen and Forkman, 2012; Cecchin, 2014), but it is not necessarily more hygienic than sawdust. In the present study, the animals kept in sawdust had a lower cleaning score, showing that the sawdust was more hygienic than sand, but little is known in the literature about the effect of the sawdust bed when compared to the sand bed in tropical conditions.

1.4. CONCLUSIONS

Sawdust may be used to replace sand in freestall beds for dairy cows with high milk yield. However, care must be taken with the management of bed replacement to ensure bed quality, since sawdust is an organic material and supports bacterial growth, especially when it is wet. Furthermore, more studies should be carried out with a longer duration and spaced out over the seasons to ensure that the beneficial effect of the sawdust bed is not lost over time.

1.5. LITERATURE CITED

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Figure 1 - Sand beds (a) and sawdust beds (b)

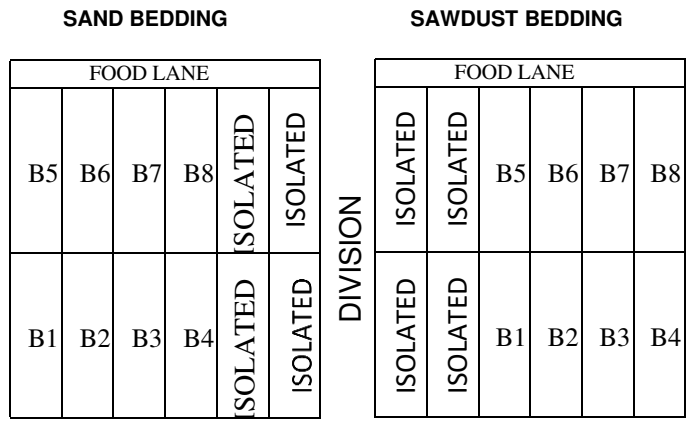


Figure 2 - Organizations of stalls for collecting material from the beds.

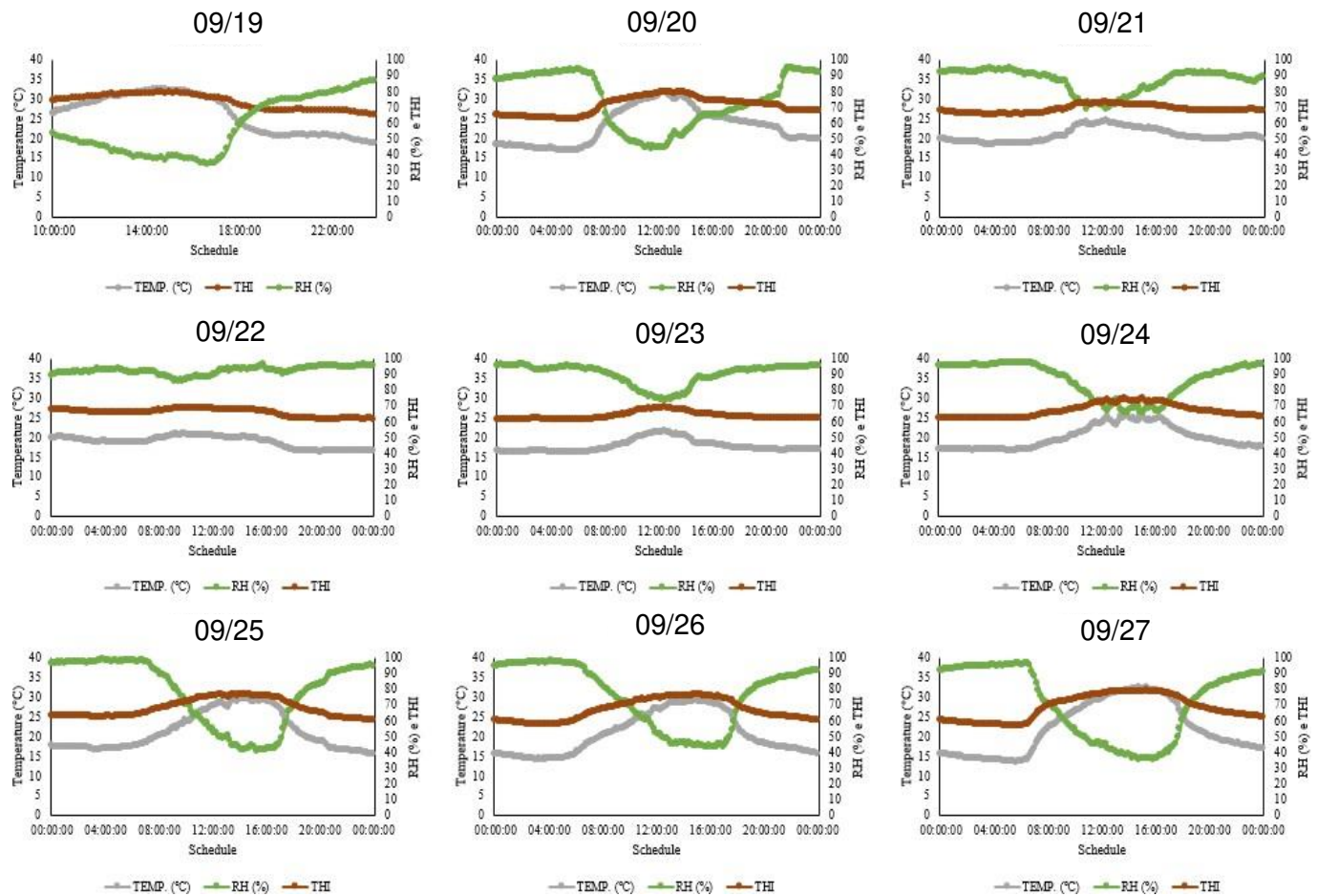


Figure 3 - Temperature graphs, relative air humidity and temperature and humidity index for the period from September 19th to 27th.

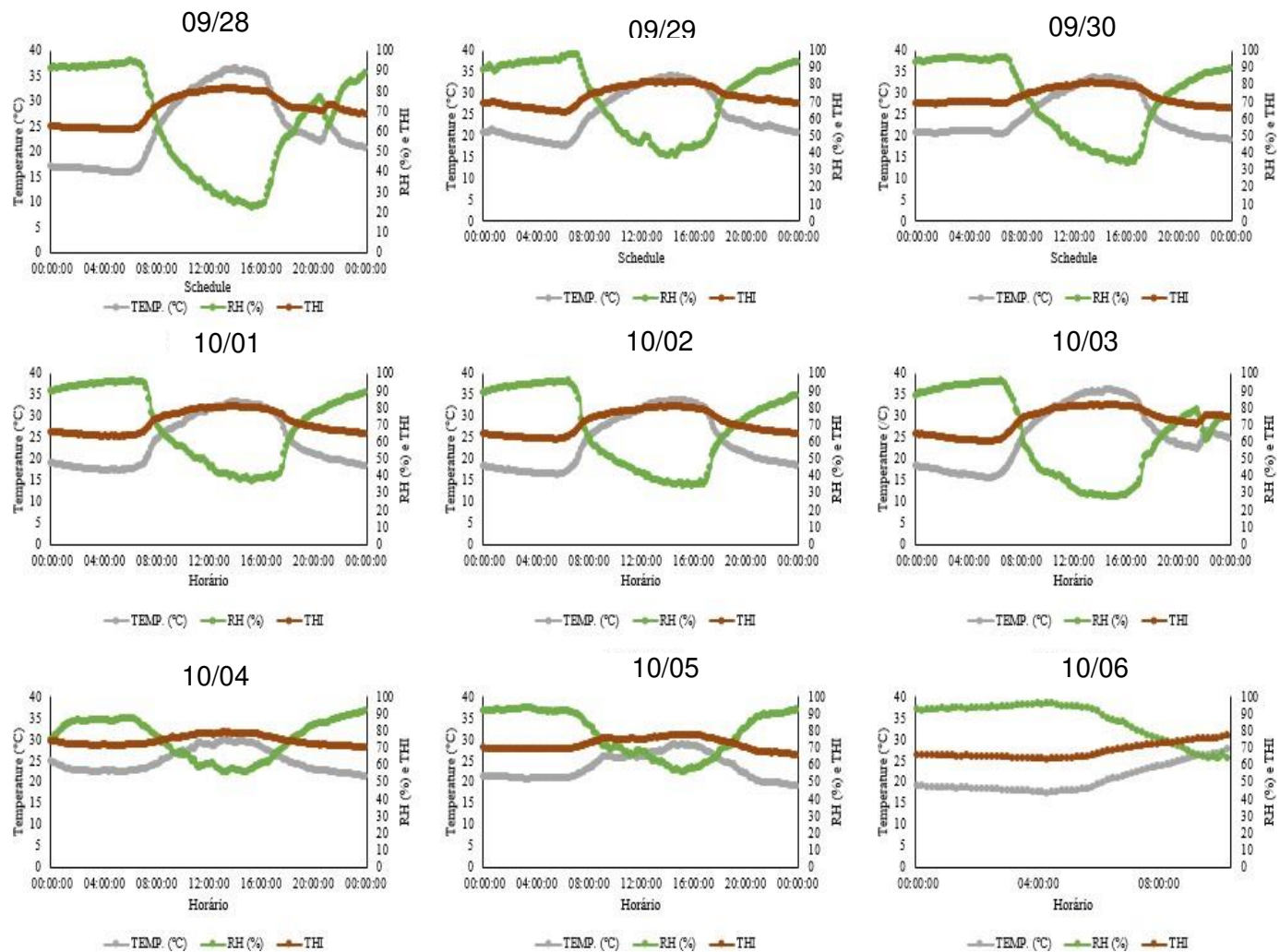


Figure 4 - Temperature graphs, relative air humidity and temperature and humidity index for the period from September 28th to October 6th.

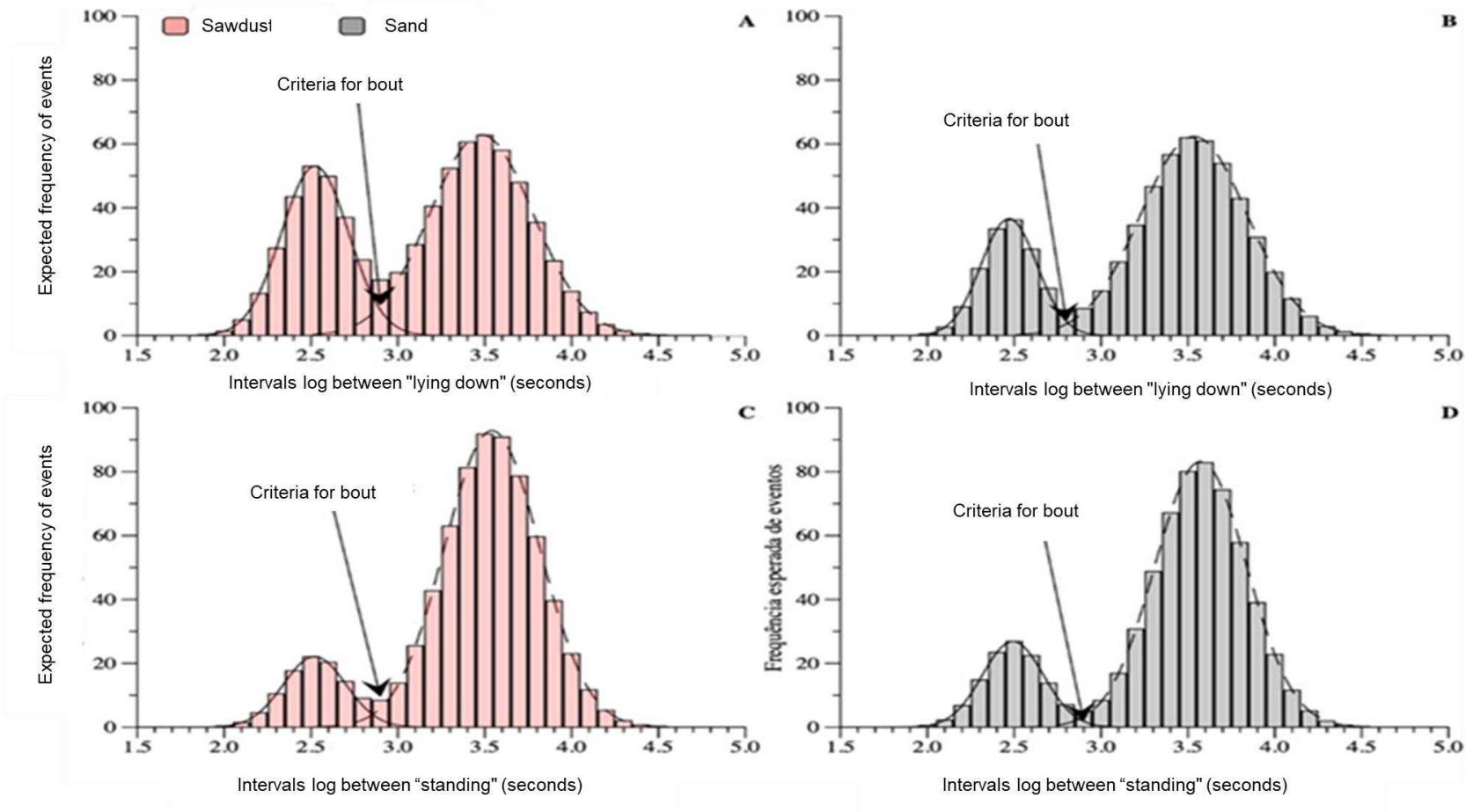


Figure 5 - Meal criteria. The intersection of the intervals of the intrameal distribution curve, and the intermeal interval distribution curve. A. Meal criteria for animals housed in sawdust bedding in lying behavior; B. Meal criteria for animals housed in sand bed in lying behavior; C. Meal criteria for animals housed in sawdust bedding in standing behavior; D. Meal criteria for animals housed in sand bed in standing behavior.

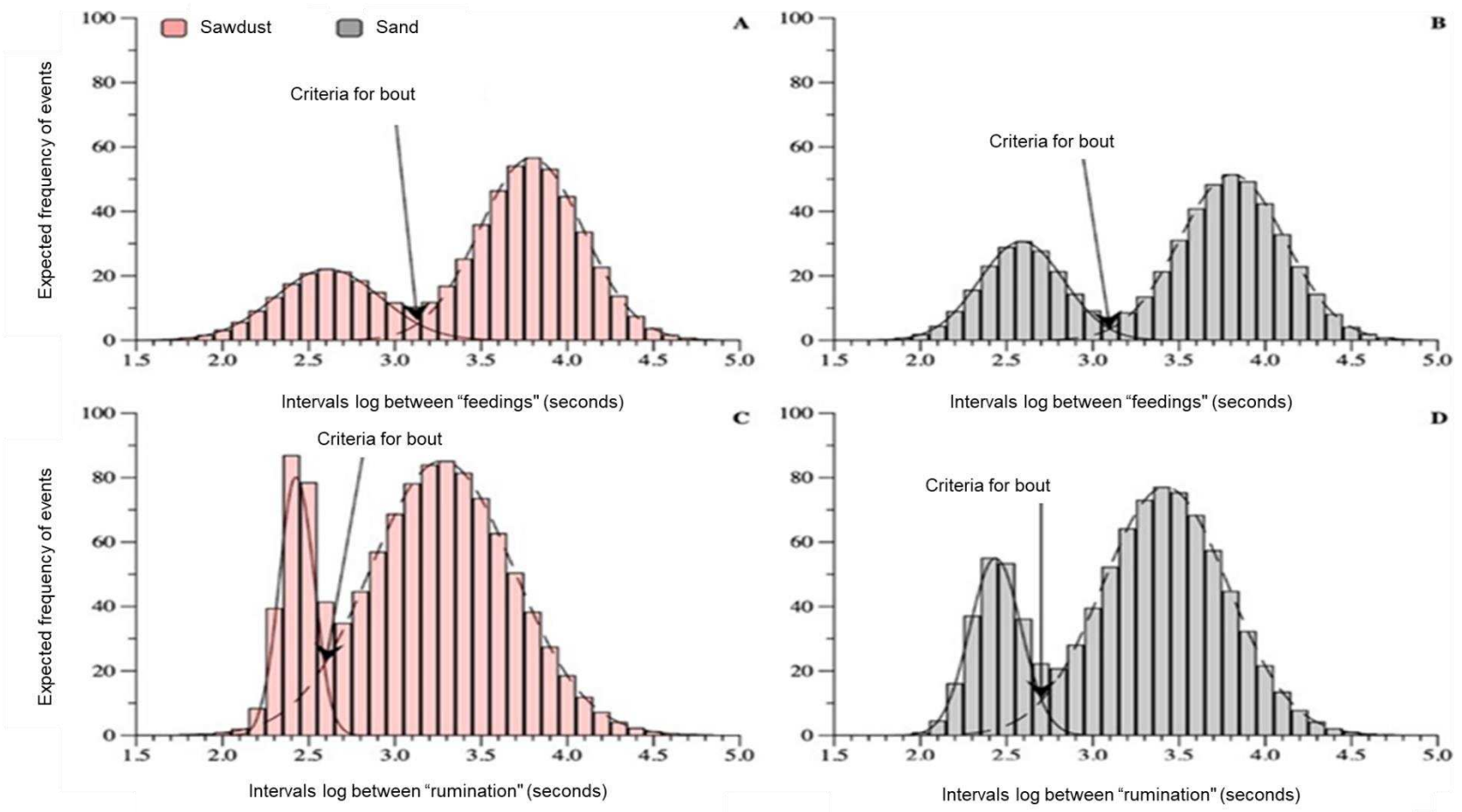


Figure 6 - Meal criteria. The intersection of the intervals of the intrameal distribution curve, and the intermeal interval distribution curve. A. Meal criteria for animals housed in sawdust bedding in feeding behavior; B. Meal criteria for animals housed in sand bed in feeding behavior; C. Meal criteria for animals housed in sawdust bedding in rumination behavior; D. Meal criteria for sand-bedded animals in rumination behavior.













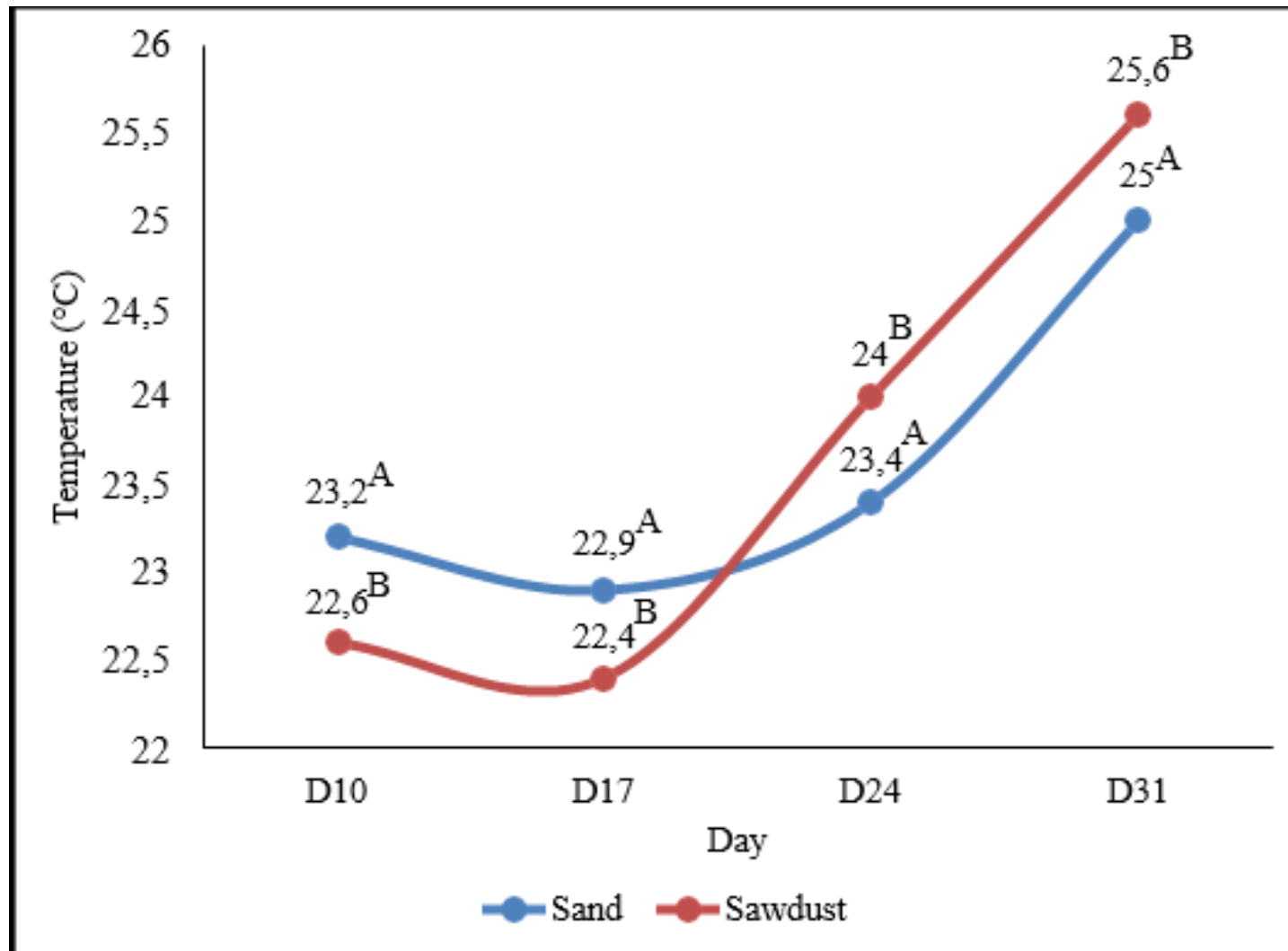
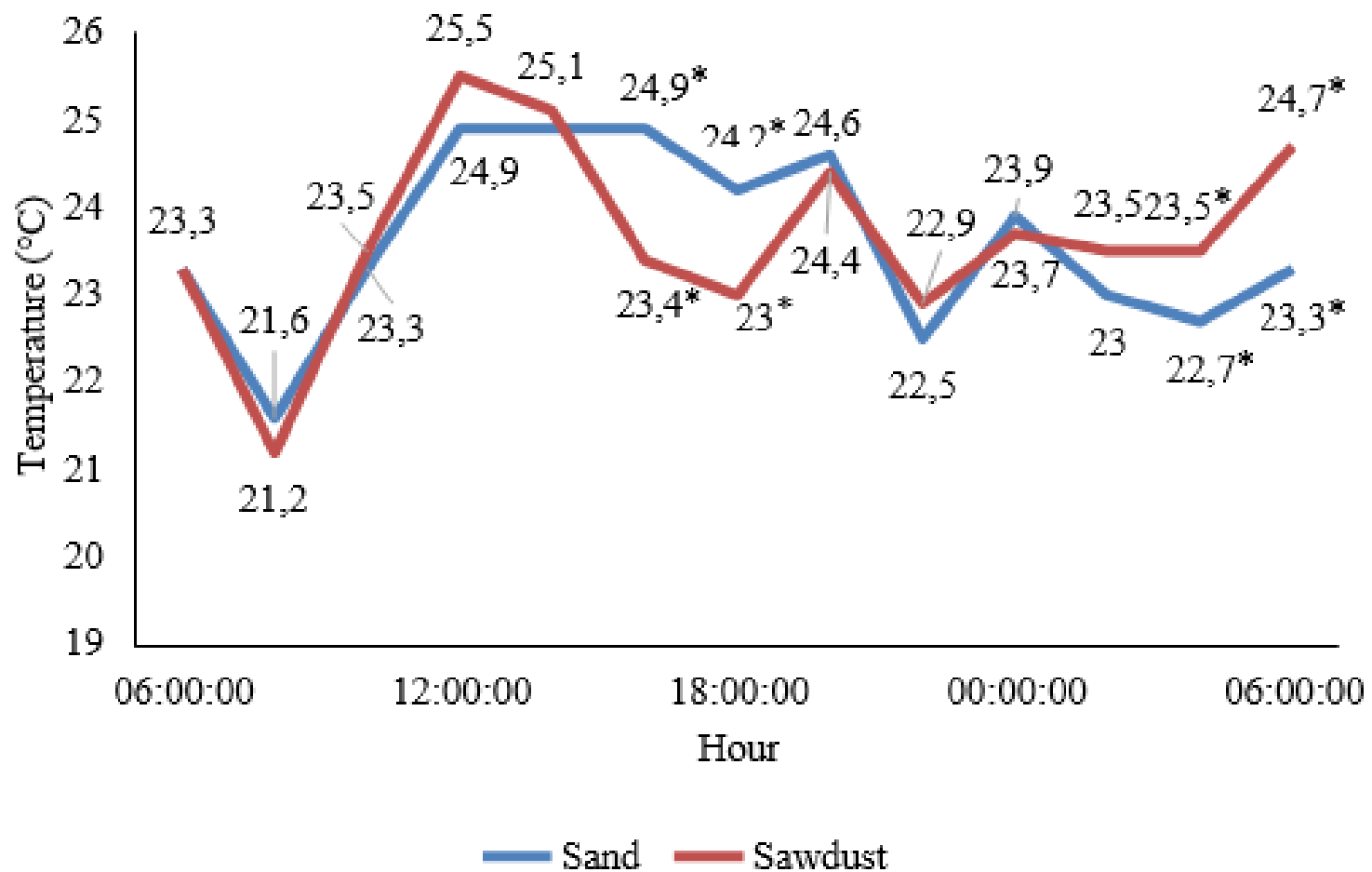
1 - Clean udder	2 - Slightly dirty udder	3 - Dirty udder	4 - Very dirty udder
			
1 - Clean thigh	2 - Slightly dirty thigh	3 - Dirty thigh	4 - Very dirty thigh
			
1 - Clean leg	2 - Slightly Dirty leg	3 - Dirty leg	4 - Very dirty leg
			

Figure 7 - Score card for cleaning score evaluation. Adapted from DOHMEN et al., 2010.



Different letters refer to statistical difference ($P < 0.05$).

Figure 8 - Average temperature values of sand and sawdust beds according to the days of evaluation.



*Refers to the statistical difference ($P < 0.05$).

Figure 9 - Total average temperature value of sand and sawdust beds distributed every two hours for a period of 24 hours.

Table 1 - Milk yield (kg/day) and SCC (cells/mL) according to the type of bedding

Item	Bed		SEM ¹	Period				P-value		
	Sand	Sawdust		P1	P2	P3	P4	Bed	Per	Bed × Per
Milk yield	39.9	42.4	2.26	40.3	41.8	41.6	41.0	0.4332	0.2288	0.1450
SCC (×10 ⁵)	1.94	2.11	0.055	1.98	2.16	2.03	1.94	0.0424	0.2409	0.2280

¹SEM = standard error of the mean.

Table 2 - Evaluation of the udder, thigh and leg cleanliness score according to a scorecard for the type of bedding

Item	Bed			Period				<i>P</i> -value		
	Sand	Sawdust	SEM ¹	P1	P2	P3	P4	Bed	Per	Bed × Per
Udder	2.8	2.5	0.17	2.31	2.56	2.87	2.75	0.30	0.29	0.40
Right thigh	2.5	2.0	0.23	2.06	2.43	2.25	2.18	0.13	0.15	0.66
Left thigh	2.6	2.0	0.16	2.20	2.25	2.56	2.18	0.01	0.39	0.88
Left and right thigh	2.6	2.0	0.18	2.15	2.34	2.40	2.18	0.04	0.33	0.68
Right leg	3.1	2.8	0.16	2.93	3.06	2.93	3.00	0.24	0.90	0.33
Left leg	3.2	2.8	0.17	2.87	3.06	3.06	2.93	0.16	0.82	0.78
Left and right leg	3.1	2.8	0.14	2.90	3.06	3.00	2.96	0.15	0.88	0.82

¹SEM = standard error of the mean.

Table 3 - Evaluation of standing and lying down behavior influenced by the type of bed.

Item	Bed		SEM ¹	P-value
	Sand	Sawdust		
Standing				
Total observed time (min/d)	499.1	582.9	46.21	0.22
<i>Meal time</i> (min/d)	926.3	840.2	45.80	0.21
<i>Meal criteria</i> (min)	3.0	3.0	0.04	0.84
N ^o events (events/d)	14.1	12.3	0.68	0.08
Average duration/event (min/event)	35.9	47.4	3.59	0.04
Average duration/ <i>meal</i> (min/d)	67.6	68.8	5.54	0.88
Lying down				
Total observed time (min/d)	813.96	720.60	46.32	0.18
<i>Meal time</i> (min/d)	604.90	701.25	45.14	0.15
<i>Meal criteria</i> (min)	2.92	2.93	0.09	0.93
N ^o events (events/d)	10.83	10.54	0.64	0.75
Average duration/event (min/event)	76.99	71.89	8.05	0.66
Average duration/ <i>meal</i> (min/d)	55.89	68.98	5.37	0.11

¹SEM = standard error of the mean.

Table 4 - Evaluation of feeding and rumination behavior influenced by the type of bed.

Item	Bed		SEM ¹	P-value
	Sand	Sawdust		
Feeding				
Total observed time (min/d)	257.3	285.3	21.61	0.37
<i>Meal time</i> (min/d)	1164.3	1122.8	19.62	0.16
<i>Meal criteria</i> (min)	3.1	3.1	0.08	0.82
N ^o events (events/d)	10.1	9.5	0.63	0.51
Average duration/event (min/event)	25.6	30.1	1.60	0.07
Average duration/ <i>meal</i> (min/d)	119.5	122.4	9.77	0.83
Ruminating				
Total observed time (min/d)	579.3	546.3	12.01	0.07
<i>Meal time</i> (min/d)	821.0	879.9	13.51	0.01
<i>Meal criteria</i> (min)	2.8	2.8	0.07	0.61
N ^o events (events/d)	17.1	15.3	0.76	0.10
Average duration/event (min/event)	34.4	36.1	1.67	0.49
Average duration/ <i>meal</i> (min/d)	48.9	58.0	2.29	0.01

¹SEM = standard error of the mean.

Table 5 - Average count of Enterobacteriaceae and Staphylococcus (log10 cfu/g) by period and type of bed.

Item	Bed			Period					P-value		
	Sand	Sawdust	SEM ¹	d 0	d 7	d 14	d 21	d 28	Bed	Period	Bed × Period
<i>Enterobacteriaceae</i>	8.20	12.30	0.75	3.57 ^C	11.01 ^B	13.64 ^A	12.60 ^{BA}	10.53 ^B	0.001	0.001	0.410
<i>Staphylococcus</i>	10.90	10.70	0.09						0.450	0.001	0.001
Sand				5.33e ^{-15Ab}	11.23 ^{Aa}	13.15 ^{Aa}	15.52 ^{Aa}	14.40 ^{Ba}			
Sawdust				8.88e ^{-15Ab}	11.63 ^{Aa}	12.76 ^{Aa}	13.57 ^{Ba}	15.73 ^{Aa}			

Lowercase letters refer to statistical difference ($P \leq 0.05$) in rows and uppercase in columns.

¹ Standard error of the mean.

CHAPTER 2 – COLOSTRUM REPLACER FOR BEEF CALVES

2.1. INTRODUCTION

After the calf is born, several procedures are performed to maintain the animal's health. One of the managements, is colostration, which is essential to transfer immunity from dam to calf, guaranteeing immunoglobulins levels that will help the immune system (Bittar & Tomaluski, 2021).

For dairy cattle there are many works and the importance of supplementation is known. But for beef cattle this is a novelty. Beef cows in unfavorable environmental conditions may have limitations in producing colostrum in quality and quantity (Souza, 2021). Thus, the importance of studying this supplementation. Furthermore, this study stands out as the first study evaluating powdered colostrum for beef cattle.

Thus, we hypothesized that calves that receive powdered colostrum supplementation will have greater immunity and lower morbidity and mortality rates. In addition, they will show greater ADG than those that receive colostrum only from dams. The aim of the study was to evaluate two levels of colostrum powder supply on the quality of colostrum efficiency, morbidity rate, mortality and ADG of beef calves.

2.2. MATERIALS AND METHODS

The experiments were carried out at Fazenda Santa Mônica, in the municipality of São João da Ponte – Minas Gerais - Brazil and at Fazenda Nelore Mocho CV, in the municipality of Presidente Venceslau - São Paulo – Brazil, following the standard procedures for animal care and handling according to the university's guidelines (108/2022).

2.2.1. Experiment I – Fazenda Santa Mônica

2.2.1.1. Animals and management

In experiment 1 we used 206 calves $\frac{3}{4}$ Angus \times Nelore, offspring from $\frac{1}{2}$ Angus \times Nelore primiparous cows divided into 3 groups:1) No supplementation group (NSG)

(n = 68): these animals received only colostrum from the dam, with no powdered colostrum supplementation;

2) IgG powder 50 g (50l) of powdered colostrum (n=68): these animals received 235 g of powdered colostrum containing 50 g IgG diluted in 500 mL of water; 3) IgG powder 100 g (100l) of colostrum powder (n=70): These animals received 470 g of powdered colostrum containing 100 g IgG diluted in 1 L of water.

The animals in the 50l group received powdered colostrum diluted in 500 mL of clean water, and the 100l group received the powdered colostrum diluted in 1 liter of clean water. Both were prepared at a temperature of 40 to 45°C. The animals in these groups received colostrum within 12 hours after birth, through an esophageal tube.

The animals were in maternity paddocks. Cows were fed a total mixed ration with corn silage, grass silage, DDG, cornmeal and vitamin mineral premix. Water was offered ad libitum. The paddock was shaded with eucalyptus to meet animal welfare conditions and reduce the animals' stress.

2.2.1.2. Analysis

The serum protein level was assessed using an optical brix refractometer, a digital brix refractometer and a g/dL refractometer. For this procedure, blood was collected from all animals participating in the experiment, within 24 to 48 hours after colostrum.

Blood collection was performed by puncturing the jugular vein with a vacuum needle in a tube containing clot activator to obtain serum. Subsequently, the sample was taken to the laboratory where they were centrifuged at 2000 x for 20 minutes at 4°C. Then the serum was pipetted and stored in *ependorfs* and then stored in a freezer for further analysis.

The animals were evaluated up to 15 days after colostrum procedure if there was an incidence of diseases (diarrhea, pneumonia and bovine parasitic sadness) and mortality in the animals in the experimental period.

A visual assessment of the health score of the animals was performed daily in order to control the sick animals and the early diagnosis of disease. For diarrhea, the assessment was made by observing the consistency of the stool (firm, pasty or

watery). For pneumonia, the following were observed: presence and frequency of cough, nasal and ocular discharge, and positioning of the ears. And for bovine parasitic sadness (BPS) the color of the ocular mucosa and if the animal presented apathy was evaluated (McGUIRK, 2008).

This observation was recorded, and according to the health sector's own monitoring system. All records were track the date of incidence and the disease diagnosed.

2.2.2. Experiment II – Fazenda Nelore Mocho CV

2.2.2.1. Animals

Were used 256 Nelore calves divided into 3 groups: 1) No supplementation group (NSG) (n=68): these animals received only colostrum from the dam, with no with powdered colostrum supplementation; 2) IgG powder 50 g (50I) of powdered colostrum (n=84): these animals received 235 grams of powdered colostrum containing 50 g IgG diluted in 500 mL of water; 3) IgG powder 100 g (100I) of colostrum powder (n=82): These animals received 470 g of powdered colostrum containing 100 g IgG diluted in 1 L of water.

2.2.2.2. Serum protein and Average daily gain

The serum protein level was assessed using an optical brix refractometer, a digital brix refractometer and a grams/dL refractometer. For this procedure, blood was collected from all animals participating in the experiment, within 24 to 48 hours after colostration.

Weighing tapes (Nasco, Fort Atkinson, WI) were provided to weigh calves. In the first 24 hours, the calves were weighed and the weight recorded and they were also weighed at weaning.

2.2.3. Statistical Analyses

For Experiment I, the data were evaluated using PROC GLIMMIX of the SAS Software (SAS University Edition). For all analyses, significance was declared when $P < 0.05$.

For Experiment II, data were evaluated using PROC GLIMMIX of the SAS Software (SAS University Edition). For all analyses, significance was declared when $P < 0.05$. For this experiment, no gender effect was analyzed because this effect was not observed in experiment 1.

2.3. RESULTS AND DISCUSSION

2.3.1. Experiment I

2.3.1.1. Serum total protein

Evaluating the colostrum efficiency, there was no effect ($P = 0,88$) to STP (serum total protein) concentrations, obtained for females and males. For NSG, 50I and 100I treatments, no effect ($P = 0.09$) was observed.

Although there was no difference between supplementation or not, colostrum efficiency was good and within ideal values. The present study is in agreement with the results presented in the USDA manual (2007) with levels considered excellent ($PT \geq 5.5\text{g/dL}$). FTIP (failure to transfer passive immunity) being one of the most important risk factors and predictors of morbidity and mortality in dairy calves (Windeyer et al., 2014). Silva et al. (2019) evaluating passive transfer in calves receiving maternal colostrum or different doses of colostrum substitute found similar results to the present study, with mean SPT values $> 5.5\text{ g/dL}$.

Evaluating the total protein using the Brix Refractometer, no effect ($P=0.08$ for treatment) was observed evaluating the doses of colostrum and all the evaluated animals presented values above the recommended ($\geq 8.4\%$), according to Deelen et al. (2014), the percentage of Brix is also highly correlated with the concentration of serum IgG, with values below 8.4% of Brix indicative of FTIP (Table 6).

2.3.1.2. Mortality, and Illness

There was no influence ($P>0.05$) of different amounts of colostrum on the mortality, diarrhea, and pneumonia (Table 1). Lago et al. (2018) found no correlation between the mortality risk rate between calves fed colostrum substitutes and maternal colostrum, a result like the present study. Evaluating the morbidity and mortality of dairy calves fed colostrum from a colostrum bank, the results found were 34.3% and 3.2%, respectively, reported in the pre-weaning period (Lombard et.al, 2020).

In the study conducted by Silva et al., (2019) no diseases were found in animals colostrated with different doses of antibodies from maternal colostrum or colostrum substitute. Silva (2019) stated that the provision of high level of immunoglobulins is intended to ensure adequate immunity, reduce the incidence of diseases in calves and, possibly increasing the potential for future production of these animals. Calves that receive a volume of 2 L or more of high-quality colostrum or have higher levels of serum IgG in the first week of life have lower morbidity and mortality than calves that receive lower levels of serum IgG or insufficient amounts of colostrum from high quality (Faber et al., 2005).

2.3.2. Experiment II

2.3.2.1. Serum total protein

There was an effect ($P = 0,02$) on serum total protein concentrations, evaluating the NSG, 50I and 100I, measured by the refractometer grams/dL, (Figure 10). According to Mason et al. (2022) in calves that suckled and received high quality colostrum, STP was greater and fewer calves had (FPT), suggesting that both pasture suckling and farmer management of colostrum feeding may work synergistically to improve passive transfer of immunity in calves, even when colostrum feeding is delayed.

Evaluating the total protein through the digital brix refractometer, an effect was observed for the treatment by evaluating the doses of colostrum administered, with the 50I treatment resulting in a higher value (Figure 11).

Evaluating the total protein through the optical brix refractometer, there was no influence ($P>0.05$) for treatments (Figure 12). The values found in the present study

are considered adequate, according to Lombard et al. (2020), the level of total protein found is considered excellent.

There was no influence ($P = 0.31$) of different amounts of colostrum on ADG (Figure 13). In a study carried out by Ahmadi et al. (2021) evaluating different doses of colostrum, with Hanwoo beef cattle, no influence was found for treatment and period.

2.4. CONCLUSIONS

The effect of colostrum supplementation was different from Nellore pure calves and $\frac{3}{4}$ Angus \times Nellore calves, with better results of passive transfer for Nellore calves. However, for dams well fed like in these experiments, no effect on morbidity, mortality and animal performance were observed for calves.

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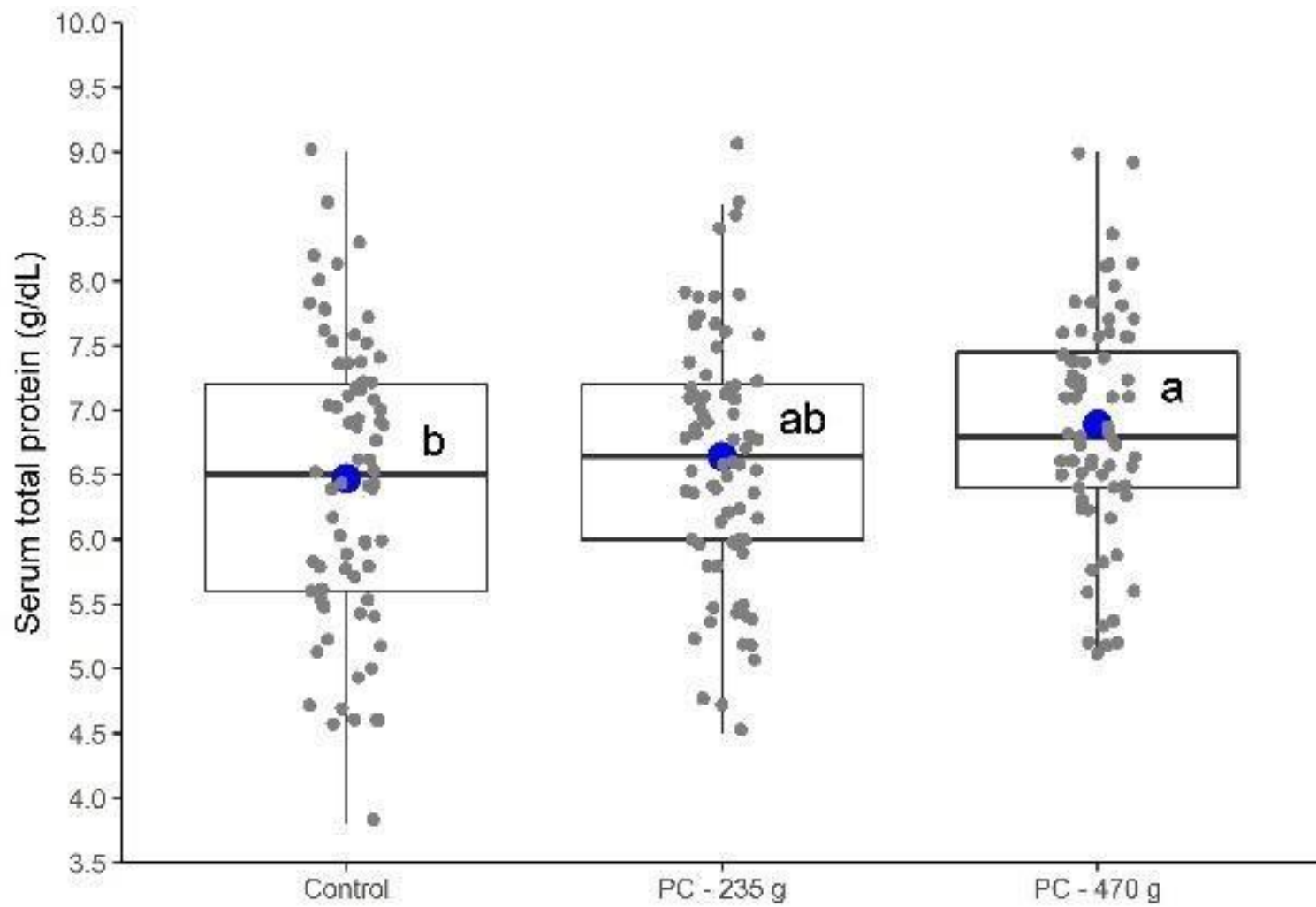


Figure 10 - Serum protein (g/dL) and treatments

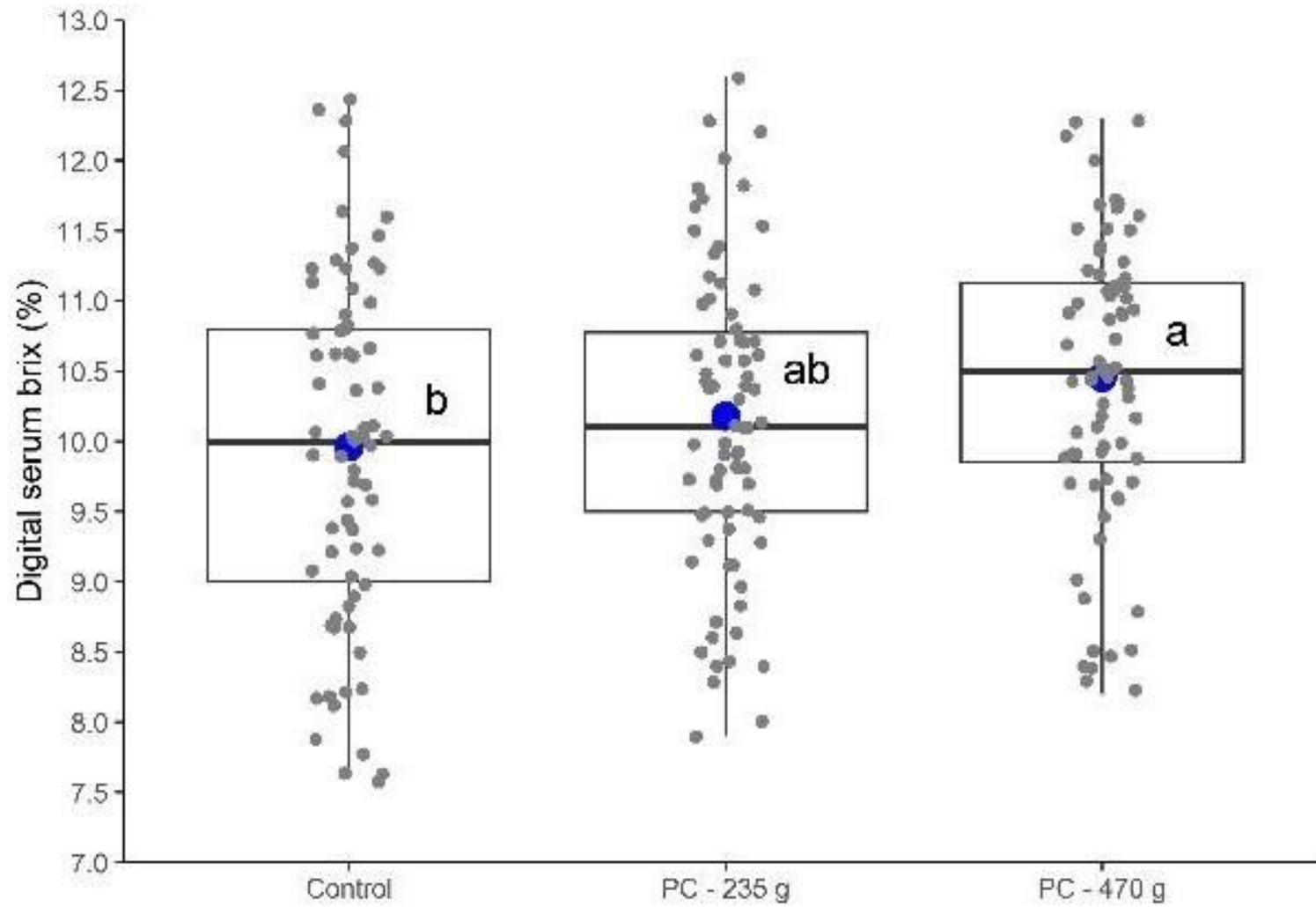


Figure 11 - Total protein with digital refractometer (%brix) and treatments

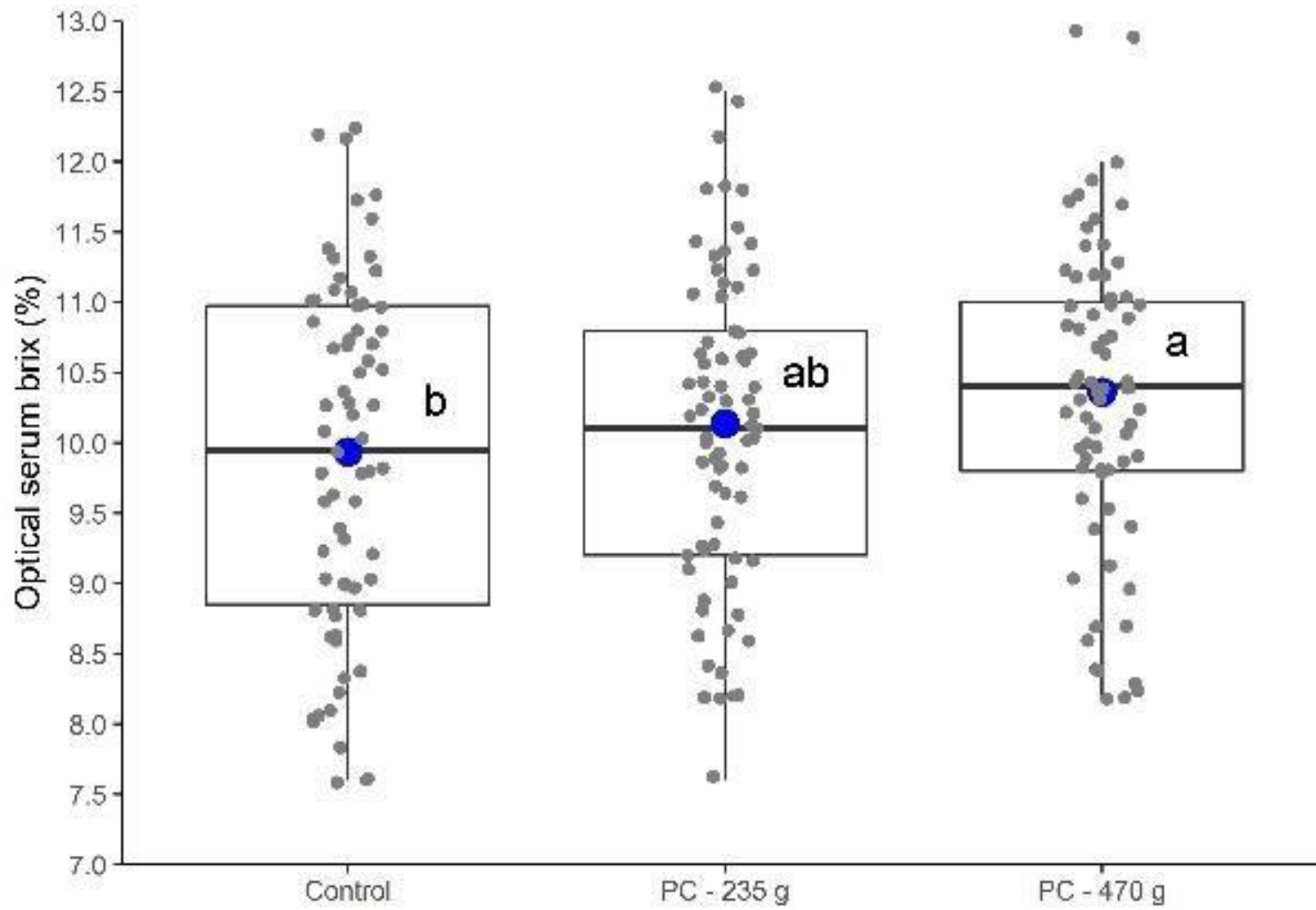


Figure 12 - Total protein with optical refractometer (%brix) and treatments

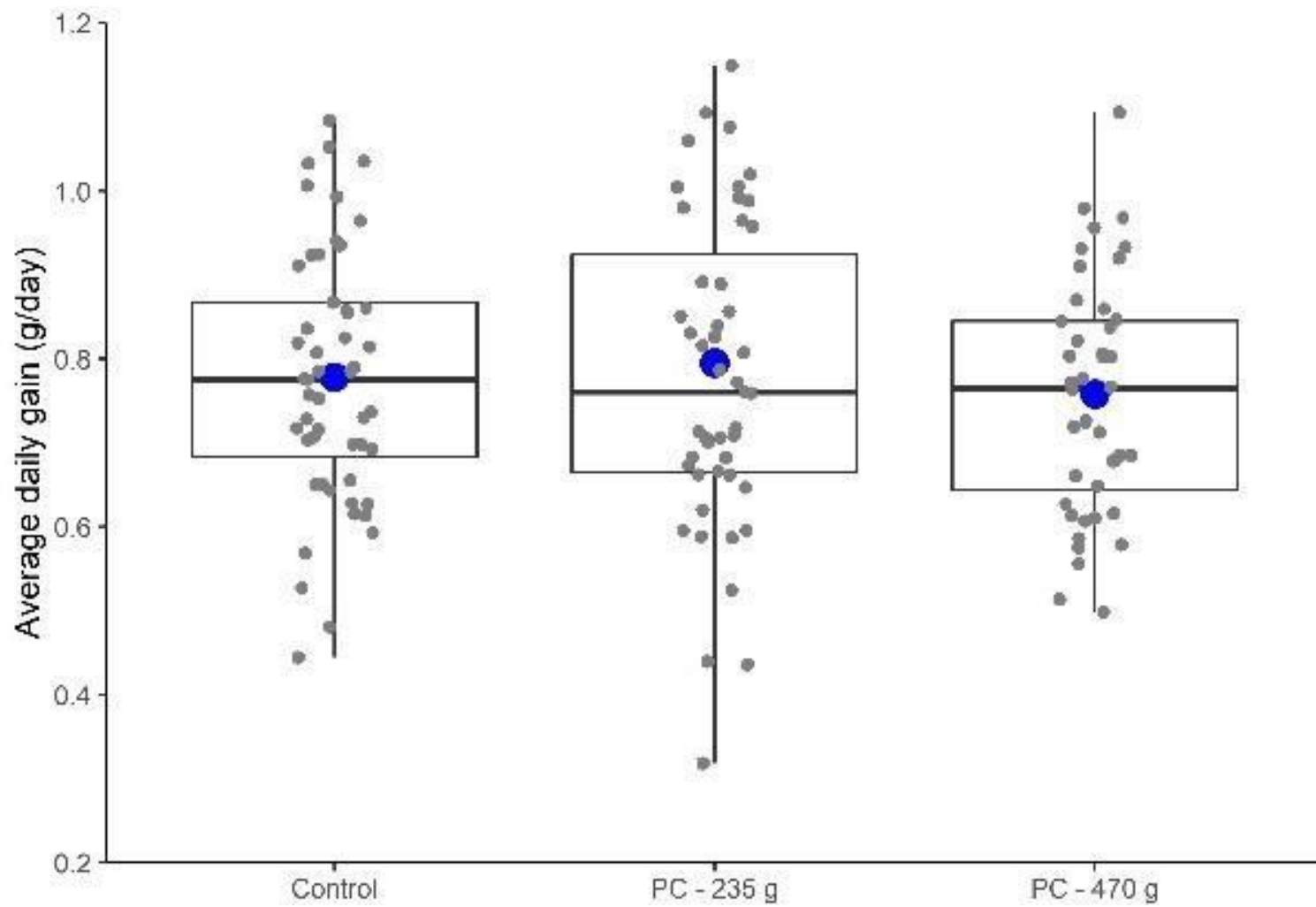


Figure 13 - Average daily gain and treatments

Table 6 - Assessment of passive immunity transfer, vitality score, mortality and disease incidence in calves that received different doses of immunoglobulins from maternal colostrum or colostrum substitute.

Parameters	Gender		Colostrum			P-value		
	Female	Male	NSG	50I	100I	Gender	Colostrum	Gender x Colostrum
Total Protein (g/dL)	6.56 ± 0.115	6.61 ± 0.092	6.81 ± 0.127	6.44 ± 0.131	6.51 ± 0.125	0.7226	0.0986	0.8751
Total Protein (brix)	9.86 ± 0.151	9.95 ± 0.121	10.21 ± 0.167	9.75 ± 0.173	9.76 ± 0.164	0.6472	0.0855	0.8946
Mortality (%)	6.91 ± 154.42	2.10 ± 0.294	8.89 ± 231.62	1.94 ± 0.384	2.68 ± 0.568	0.9752	0.5463	0.2098
Diarrhea (%)	1.29 ± 0.277	1.38 ± 0.224	1.29 ± 0.300	1.44 ± 0.331	1.27 ± 0.294	0.8001	0.9197	0.8276
Pneumonia (%)	2.85 ± 0.503	3.23 ± 0.490	3.61 ± 0.734	2.88 ± 0.556	2.62 ± 0.486	0.5743	0.5203	0.6315