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Effects of different production systems on health, zootechnical and metabolic parameters in Holstein calves in the extreme south of Brazil

Efeito de diferentes sistemas de produção sobre parâmetros de saúde, zootécnicos e metabólicos de bezerras holandês no extremo sul do Brasil

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Highlights ____

Dairy heifer rearing system implemented on a property in southern Brazil. Heifers raised in a collective system had a lower incidence of diarrhea. Heifers raised in an individual system had a lower incidence of bronchopneumonia. Heifers raised collectively showed higher weight gain and thoracic perimeter.

Abstract _

The aim of this study was to evaluate the incidence of diseases as well as metabolic and zootechnical parameters in Holstein calves maintained in individual or collective housing systems in the extreme south of Brazil. One hundred calves were included immediately after birth and monitored for 90 days. Animals were randomly divided into two groups: individual stalls group (ISG; n=50) and collective stalls group (CSG; n=50). ISG calves were individually housed in 1.2m² wood pens and received 6 L of milk/ day and concentrate food and water *ad libitum* for 90 days. Calves in the CSG were housed in groups of up to 25 animals, in a total of two stalls containing one animal per 3 m². CSG calves received up to 8 L of milk/day at 0-15 days, milk *ad libitum* at 15-40 days, 7.2 L of milk. /day at 40-60 days, and gradual weaning at 60-90 days with concentrate food and water *ad libitum*, using an automatic feeder. In both groups, Zootechnical parameters (weight, thoracic perimeter, height at the withers, and rump width) were measured weekly during the first 28 days. Metabolic parameters evaluated in this study were total

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calcium, beta-hydroxybutyrate (BHBA), free fatty acids (FFA), and glucose. The animals were monitored daily for clinical signs of diseases diarrhea and respiratory symptoms. General morbidity was higher in CSG 92% compared to ISG 74% (p<0.05). We observed increased diarrhea-related morbidity and a higher rate of recurrence in the ISG (50% and 60%, respectively; p<0.05) compared to those in the CSG (both 20%, p<0.05 between groups). The morbidity due to bronchopneumonia was higher in the CSG (92%) than in the ISG (74%, p<0.05). Recurrence of bronchopneumonia and mortality were similar between groups (p>0.05). Glucose, FFA, and BHBA concentrations were higher in the ISG (p<0.05) and total calcium higher in CSG (p<0.05). Finally, the weight and thoracic perimeter were higher in the CSG than those in the ISG, whereas the height and, rump width, at the withers was higher in the ISG than that in the CSG (p<0.05). In conclusion, animals raised in a collective system with automatic feeders exhibited less diarrhea and increased serum calcium as well as improved zootechnical development, although they had a higher incidence of bronchopneumonia.

Key words: Bovine neonatology. Growth. Diarrhea. Bronchopneumonia.

Resumo -

O objetivo deste estudo foi avaliar a incidência de doencas, os parâmetros metabólicos e zootécnicos em bezerras Holandesas mantidas em sistemas de alojamento individual ou coletivo no extremo sul do Brasil. Cem bezerros foram incluídos imediatamente após o nascimento e acompanhados por 90 dias. Os animais foram divididos aleatoriamente em dois grupos: grupo baias individuais (ISG; n=50) e grupo baias coletivas (CSG; n=50). Bezerros ISG foram alojados individualmente em baias de madeira de 1,2 m² e receberam 6 L de leite/dia, ração concentrada e água ad libitum por 90 dias. Os bezerros no CSG foram alojados em grupos de até 25 animais, em um total de duas baias contendo um animal por 3 m². Bezerros CSG receberam até 8 L de leite/dia aos 0-15 dias, leite ad libitum aos 15-40 dias, 7,2 L de leite/ dia aos 40-60 dias e desmame gradual aos 60-90 dias com alimento concentrado e água ad libitum, usando comedouro automático. Em ambos os grupos, os parâmetros zootécnicos (peso, perímetro torácico, altura na cernelha e largura da garupa) foram medidos semanalmente durante os primeiros 28 dias e uma vez a cada 15 dias até o desmame. Amostras de sangue foram coletadas semanalmente durante os primeiros 28 dias. Os parâmetros metabólicos avaliados neste estudo foram cálcio total, betahidroxibutirato (BHBA), ácidos graxos livres (AGL) e glicose. Os animais foram monitorados diariamente quanto a sinais clínicos de doenças, diarreia e sintomas respiratórios. A morbidade geral foi maior no CSG (92%) em comparação com o ISG (74%; p<0.05). Observou-se aumento da, relacionada à diarreia e maior taxa de recorrência no ISG (50% e 60%, respectivamente; p<0,05) em comparação com o CSG (ambos 20%, p<0,05 entre os grupos). A morbidade por broncopneumonia foi maior no CSG (92%) do que no ISG (74%, p<0,05). A recorrência da broncopneumonia e a mortalidade foram semelhantes entre os grupos (p>0,05). As concentrações de glicose, AGL e BHBA foram maiores no ISG (p<0,05) e cálcio total maior no CSG (p<0,05). Finalmente, o peso e o perímetro torácico foram maiores no CSG do que no ISG, enquanto a altura e largura da garupa na cernelha foi maior no ISG do que no CSG (p<0,05). Em conclusão, os animais criados em sistema coletivo com comedouros automáticos apresentaram menos diarreia e aumento do cálcio sérico, bem como melhor desenvolvimento zootécnico, embora tenham apresentado maior incidência de broncopneumonia.

Palavras-chave: Neonatologia bovina. Crescimento. Diarreia. Broncopneumonia.

Introduction _____

There are different rearing systems available for dairy calves. Regardless of the system choice, it is crucial to ensure an environment with minimal infective pressure to prevent the spread of diseases among neonates (Gaspar et al., 2016). Several factors affect calf development; however, which system has a greater impact on health and development remains unclear. Furthermore, passive immunity, hygiene, and welfare are closely associated with the main neonatal diseases affecting dairy calves (Costa et al., 2016).

Calves are particularly susceptible to infectious diseases due to their developing immune system (United States Department of Agriculture [USDA], 2017). According to Paranhos da Costa & Silva (2014), good practices for calf rearing should be applied and can reduce in 70% and 54% the mortality and antibiotic treatments due to infectious diseases, respectively. Palczynski et al. (2021) demonstrated that management of rearing practices should focus on technical solutions, as well as infrastructure, health, and welfare of dairy calves. This highlights the importance of disease control measures within the chosen system including immunological support and adequate supplementation with colostrum, adequate diet, vaccination, hygiene, and biosafety measures to control pathogens in the environment (Johnson et al., 2021).

In that way, the use of automated feeding systems in group calf rearing has increased since these systems improve working conditions, reduce labor requirements, and facilitate feeding large volumes of milk in several portions throughout the day (Medrano-Galarza et al., 2017). Group-managed calves have greater socialization, increased dry matter intake and faster growth compared to calves raised in isolation (Costa. et al., 2016). Moreover, in group rearing systems with automatic feeders, milk intake is increased and more frequent with greater control over the intake amounts (Lopes et al., 2019). Furthermore, social isolation can have a negative impact on social and feeding behaviors as well as on cognitive development (Hötzel et al., 2014).

Nevertheless, some farmers attribute greater disease incidence to the automatic feeders and to the group housing systems due to increased contact between animals (Medrano-Galarza et al., 2017). The risk of respiratory and enteric diseases is significantly higher among calves reared in groups of 6-30/pen with automatic feeders compared to those fed manually, for resulting in closer animal-to-animal contact, which could promote the spread of infections (Lundborg et al., 2005; Svensson et al., 2003).

Evaluating metabolic parameters can provide important information regarding the nutritional status of cattle. Serum glucose levels can be used to determine the energetic status of calves (Hayashi et al., 2006). Furthermore, beta-hydroxybutyrate (BHB) and free fatty acids (FFA) are related to the rate of lipid reserve mobilization during a negative energy balance (González & Scheffer, 2002). Calcium maintains bone structural integrity and regulates ion concentrations for various physiological functions, playing an important as a signal and activator of immune cells (Rosol & Capen, 1997). Additionally, metabolic parameters can be evaluated to predict body development (Aghakhani et al., 2023) and diseases (Murray et al., 2014).

Material and Methods _____

Experimental design

This study was approved by the Ethical Committee on Animal Experimentation of the *Universidade Federal de Pelotas* registered under number 0436. The experiment was conducted at a farm using the intensive milk production system located in Rio Grande, RS, Brazil (32° 16 'S, 52° 32' E).

The calves were fed 4 L of colostrum in the first 24 h after birth. The colostrum quality was evaluated using а brix refractometer, and only colostrum with a quality above 21% was used (Godden et al., 2019; Lombard et al., 2020). A blood sample was obtained from all calves at 24-48 h after birth via jugular vein puncture using vacuum tubes with ethylenediaminetetraacetic acid (EDTA) to evaluate passive immunity. Plasma was separated to measure total plasma protein (TPP) using a refractometer. Adequate passive immunity transfer was determined for animals exhibiting TPP > 5.5 g/ dL (Tyler et al., 1999). Animals with TPP below this threshold value were removed from the study.

One hundred calves were selected and identified with numbered earrings according to the farm's protocol. After birth, they were taken to the place where they would be raised, either in individual stalls or in a collective system. The animals were randomly divided into two groups with according to the rearing systems: individual stall group (ISG, n=50), the animals were kept in a 1.2m² wooden enclosure and fed with 6 L of milk/day, in addition to concentrate and water ad libitum for 90 days; group collective stalls (GCS, n=50), the animals were kept in individual stalls during the first 15 days of life and then transferred to the collective system with a spacing of one animal per 3 m2, where they were divided into two stalls with a maximum of 25 animals each (Stall 1: calves from 15 to 50 days old; Stall 2: calves from 51 to 90 days old). The stalls were divided by iron bars, not preventing contact between the two age groups. Animals were fed using the Calf Feeder system (CF500S, DeLaval International AB, Tumba, Suécia). In the first 20 days after birth, animals received 8 L of milk/day; from 20-40 days milk was offered ad libitum; from 40-60 days calves received 7.2 L of milk/day; from 60-90 days calves were gradually weaned with an incremental reduction in the milk volume over time. The CSG animals also received concentrate and water ad libitum. Additionally, the CSG calves were submitted to instrumental music during the experimental period.

Blood sampling and metabolic evaluations

Samples were obtained from 20 animals in each group in 1 (24-48 hours), 7, 14, 21, and 28 days old, randomly. Blood sampling was performed in the morning using tubes with potassium fluoride and EDTA and tubes without anticoagulant. Blood was centrifuged to separate serum and plasma and stored at -20°C. Using the serum we measured total calcium concentration, BHBA, and FFA. The plasma samples collected in tubes with potassium fluoride were used to measure glucose concentrations. All the analyses were carried out using the biochemical analyzer Labmax Plenno (Labtest Diagnóstica SA, Brazil) according to the manufacturer's instructions.

Disease occurrence

Daily clinical monitoring allowed diagnosis of scours and bronchopneumonia, aswellasotherdiseases.Todiagnosediarrhea, clinical signs such as fever, inappetence, dehydration, and fecal score were evaluated (Lorenz et al., 2011). The fecal consistency score was classified on a 0-4 scale (0 = normal, 1 = soft, 2 = runny, 3 = profuse watery, 4 profuse watery and bloody) (Mcquirk, 2008). Animals exhibiting fecal consistency scores of 2-4 were diagnosed with diarrhea. To diagnose bronchopneumonia clinical signs such as cough, nasal and ocular secretions, dyspnea, and abnormal breathing sounds were evaluated (Riet-correa et al., 2007). The following indices were calculated based on these analyses: morbidity (number of sick animals/total number of animals), mortality (number of deceased animals/total number of animals), lethality (number of deceased animals/total number of sick animals), recurrence (number of animals falling ill twice/ number of animals falling ill at least once). Treatments were provided according to each clinical case and the protocol established in the property by the Veterinarian.

Zootechnical parameters

Animals were evaluated at birth and weekly for 28 days after birth. Subsequently, animals were evaluated on days 45, 60, and 90. Body weight was assessed using a weighing tape. Furthermore, the rump width, and thoracic perimeter were determined using a measuring tape. The height at the withers was determined using a ruler (cm). Thoracic perimeter was measured as the circumference of the thorax, height at the withers was the length from the ground to the scapular junction in a standing animal, and rump width was the length between the ischial tuberosities.

Statistical analyses

The data were analyzed using JMP (SAS, Institute Inc). The averages were analyzed using the repeated measures method considering the group, sample time point and the interaction between them. Individual means were compared using the Tukey-Kramertest.Categoricalvariableswere evaluated using the chi-square test. The odds ratio for the occurrence of diseases between groups was calculated. The experimental design adopted was completely randomized. For the analysis of disease occurrence and deaths, all 100 calves were considered; for zootechnical analysis, animals that died were excluded, totaling 91 animals (CSG n=49; ISG n=42). For metabolic analysis, 20 animals from each group were used. P-value < 0.05 was considered statistically significant.

Results and Discussion _____

All 100 animals included in the study reached the passive immunity threshold with TPP above 5.5 g/dL.

The results regarding the occurrence of disease are presented in Table 1. The morbidity and recurrence of diarrhea were higher in the ISG than those in the CSG (p<0.05). The calves in ISG were 4 times more likely to experience diarrhea than animals in CSG, and were 6 times more likely to experience recurrence of diarrhea. Conversely, the morbidity due to



bronchopneumonia was higher in the CSG than that in the ISG (p<0.05). The calves in CSG were 4 times more likely to experience bronchopneumonia than animals in ISG. Mortality and lethality of both diseases and the recurrence of bronchopneumonia were similar between the groups (Table 1). General morbidity and mortality are also presented in Table 1; in addition to deaths from diarrhea and bronchopneumonia, 4 deaths from ruminal tympany were also observed, all of them occurred in the ISG group.

Table 1

Morbidity, mortality, lethality and recurrence (n/n total) of diarrhea and bronchopneumonia in Holstein calves maintained in individual or collective housing systems in the extreme south of Brazil (n=100).

Parameter –	Groups		
	Collective	Individual	p
General morbidity %	92.0 (46/50)	74.0 (37/50)	<0.05
Morbidity for diarrhea %	20.0 (10/50)	50.0 (25/50)	<0.05
Morbidity for bronchopneumonia %	92.0 (46/50)	74.0 (37/50)	<0.05
General mortality %	2.0 (1/50)	4.0 (8/50)	>0.05
Mortality for diarrhea %	0.0 (0/50)	2.0 (1/50)	0.31
Mortality for bronchopneumonia %	2.0 (1/50)	6.0 (3/50)	0.31
Lethality for diarrhea %	0 (0/10)	0.4 (1/25)	0.85
Lethality for bronchopneumonia%	0.2 (1/46)	0.8 (3/37)	0.46
Recurrence for diarrhea %	20.0 (2/10)	60.0 (15/25)	<0.05
Recurrence for bronchopneumonia %	52.2 (24/46)	64.9 (24/37)	0.24

The data were analyzed using JMP (SAS, Institute Inc). Categorical variables were evaluated using the chi-square test. A p-value <0.05 was considered statistically significant.

In this study we observed an increased morbidity and recurrence of diarrhea in the ISG compared to that in the CSG. These data are consistent with the results by Hänninen et al. (2005), which demonstrated an improvement in the intestinal health of calves reared in collective systems. However, other studies showed that animals reared in individual wood pens exhibited a decreased incidence of diarrhea (Svensson et al., 2003). The collective system in our study had automatic feeders that maintained

a consistent temperature and volume of milk provided to the calves (James & Machado, 2013). Conversely, manual feeding hinders the precise control of the temperature and volume of milk ingested by the calves. This difference could explain the increased incidence of diarrhea in the ISG (Bittar, 2016).

Animals in the ISG exhibited a lower incidence of bronchopneumonia than those in the CSG (Hulbert & Ballou, 2012). In our study, calves in the CSG had different ages, ranging from 15-90 days old, which could be a risk factor for the development of bronchopneumonia, as shown by Maier et al. (2019), which found that calves housed with older animals were twice as likely to develop pneumonia. Moreover, our results may be related to several factors including the use of the same feeder, infective pressure, and high density of animals per pen. These factors could increase the number of pathogens and environmental stressors affecting calf health (Lago et al., 2006). The data in our study is consistent with that in the study by Brscic et al. (2012), which demonstrated an increased risk of nasal secretion in animals housed together in a large space. In a smaller space, calves reduce their activity, thus decreasing

the amount of dust in the environment and the risk of respiratory diseases. According to Lorenzo et al. (2016), calves reared individually exhibited improved lung immunity and decreased eosinophil infiltration, agreeing with the lower occurrence of bronchopneumonia in the individual system in our study.

The zootechnical evaluations showed that animals in the CSG had increased weight (Figure 1) and thoracic perimeter compared to those in the ISG (Table 2; p<0.05). Conversely, the height at the withers and rump width was higher in the ISG than in the CSI (Table 2; p<0.05).

Table 2

Zootechnical evaluations (mean ± standard error) of Holstein calves maintained in individual or collective housing systems in the extreme south of Brazil (n=91).

Parameter	Groups		
	Collective	Individual	ho
Height at the withers	80.53±0.17	82.68±0.18	<0.01
Thoracic perimeter	86.57 ± 0.22	85.10 ± 0.22	<0.01
Rump width (cm)	21.80 ± 0.13	23.46 ± 0.13	<0.01

The data were analyzed using JMP (SAS, Institute Inc). The averages were analyzed using the repeated measures method. Individual means were compared using the Tukey-Kramer test. A p-value <0.05 was considered statistically significant.

The zootechnical evaluations data demonstrated that the CSG animals had increased weight gain and thoracic perimeter compared to the ISG animals. These data are consistent with other studies showing increased weight gain in calves reared in group systems (Jensen et al., 2015). According to Albright and Arave (1997), calves that remain with the cow ingest milk 7-10 times/day on average, which results in

increased milk consumption and weight gain. In spite of this, most farmers provide limited amounts of milk to the calves due to the perception that increased milk consumption increases the incidence of food-borne diarrhea, reduces the consumption of dry feed resulting in decreased weight gain after weaning, and also increases the overall costs associated with nutrition (Jasper & Weary, 2002). Nonetheless, our data demonstrated



that maintaining the volume and temperature of milk consistent during a set period in the calf's life decreases the occurrence of diarrhea resulting in increased weight gain. Besides, the automatic milk feeders simulate the natural aspects of the feeding behaviors between the calf and the cow.

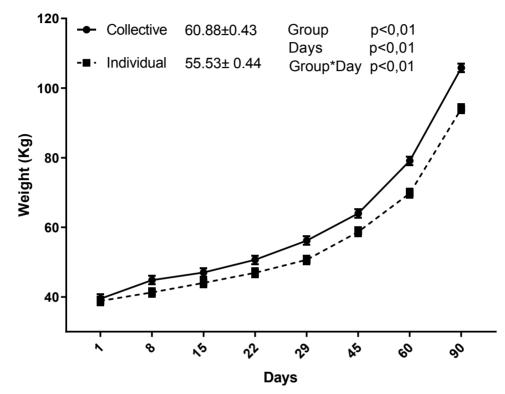


Figure 1. A-Body weight (Kg) of Holstein calves reared in different systems for 90 days after birth (n=91).

Data are presented as mean \pm standard error. The data were analyzed using JMP (SAS, Institute Inc). The averages were analyzed using the repeated measures method. Individual means were compared using the Tukey-Kramer test. A p-value <0.05 was considered statistically significant.

Serum metabolic parameters are presented in Table 3. The glucose, FFA, and BHBA concentrations were higher in the ISG (p<0.05), whereas total calcium was greater in the CSG group (p<0.05).



Table 3

Metabolic parameters (mean ± standard error) of Holstein calves maintained in individual or collective housing systems in the extreme south of Brazil (n=40).

Parameter	Groups		
	Collective	Individual	p
Glucose (mg/dL)	71.58±1.86	91.66±1.84	<0.05
Total Calcium (mg/dL)	8.35 ± 0.25	6.60 ± 0.25	<0.05
FFA (mmol/L)	0.19 ± 0.015	0.27 ± 0.014	< 0.05
BHBA (mmol/L)	0.06 ± 0.004	0.10 ± 0.003	<0.05

FFA, free fatty acids

BHBA, β-hydroxybutyrate

The data were analyzed using JMP (SAS, Institute Inc). The averages were analyzed using the repeated measures method. Individual means were compared using the Tukey-Kramer test. A p-value <0.05 was considered statistically significant.

Serum glucose concentration is the most affected biomarker during ruminal development (Ferronato et al., 2022). During the suckling in calves phase the liver shifts from using glucose to short-chain fatty acids as energy precursors (Vi et al., 2004), which can decrease serum glucose concentrations. Also, diarrhea can affect glucose levels during this transition. Montgomery et al. (2009) found glucose levels lower than normal in heifers treated for diarrhea. Moreover, stress and cortisol levels can affect glucose metabolism, and animals reared individually may undergo increased stress which can also explain the glucose results in our study (Sapolsky et al., 2000).

In our study, increased plasma concentrations of BHBA and FFA were detected in the ISG compared to those in the CSG. Reece et al. (2015) suggested that FFA are mobilized to maintain homeostasis when other energy sources are not readily available. This occurs early-on when calves are more dependent on milk as their main energy source. The higher energy mobilization required to maintain homeostasis in the ISG could be explained by the increased incidence of diarrhea in this group (Wiese et al., 2013). In our study, the ISG animals exhibited higher FFA concentrations than CSG animals. These data are consistent with those of Larson-Peine et al. (2022) and may be indicative of heightened stress and a subsequent mobilization of energy reserves.

In our study, animals in the CSG group exhibited increased calcium concentrations compared to those in the ISG group. This may be related to the possible increase in milk availability for the first one (Marcato et al., 2020). Furthermore, the reduced calcium concentrations in the ISG may be due to an increased incidence and recurrence of diarrhea in this group, increasing its loss in feces (Santos et al, 2002)

The data in this study suggests that group rearing systems in the south of Brazil result in an increased incidence of respiratory diseases and decreased incidence of diarrhea among calves compared to individual rearing. The overall mortality rate in our study was low and similar



to the risk reported in farms using individual rearing systems in Ontario and Minnesota (Windeyer et al., 2014) as well as farms using collective systems in Minnesota and in the northwest of Iowa and Wisconsin (Jorgensen & Endres, 2016). The climate in these regions varies and is classified as humid continental in Ontario, Canada (Climate-Data). This climate is characterized by strong seasonal differences, similar to that in the south of Brazil (Reboita & Krusche, 2018).

Conclusion _____

Group-reared calves in the southern region of Brazil exhibited improved metabolic and zootechnical parameters compared to those reared individually. Nevertheless, the most prevalent diseases differed among groups, with diarrhea affecting mostly animals reared individually, and bronchopneumonia affecting those reared collectively.

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