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# Clinical physiological parameters of Holstein calves in the first month of life



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## **ABSTRACT**

In the postnatal period, neonatal adaptation in terms of cardiovascular, respiratory, metabolic, thermoregulatory, and immunological functions is required, thus demanding the establishment of baseline parameters for research on neonatal calves. Few longitudinal studies have presented a set of physiological reference values for neonatal calves. The objective of this study was to evaluate physical, haematological, and biochemical parameters in young Holstein heifers in order to obtain useful information on their neonatal adaptation. Twenty-eight healthy young Holstein heifers were assessed for the collection of blood samples by jugular venipuncture at the following time points: immediately after birth, and before colostrum intake (first day of life is D1), and D2, D7, D14 and D28 days of life. Two hours prior to morning milk feed, calves were sampled, after being physically examined to establish reference values for heart rate (HR), respiratory rate (RR), and rectal temperature (RT). Several changes in physical, haematological, and biochemical parameters, secondary to neonatal adaptation, were detected. The reduction in HR and RR over time represented the maturation of the cardiovascular and respiratory systems, respectively, and the increase in RT represented the development of thermoregulatory mechanisms. Colostrum intake was reflected in several parameters, including immunoglobulin absorption and alteration of the serum protein profile. In addition, changes in glucose and cholesterol concentrations reflected the activation of the calf's metabolism. Changes in white blood cell parameters, such as an increase in lymphocyte count and decrease in neutrophil count, were associated with maturation of the immune system and the influence of cortisol levels at parturition, respectively. Changes in the red blood cell count parameters could be attributed to the replacement of erythrocytes from the foetal circulation. Variations in the physiological parameters of calves were observed during the first month of life; it is necessary to compare them with established age-specific reference ranges for a better clinical interpretation.

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# Implications

The transition to extrauterine life is characterised by clinical, haematological, and biochemical changes; therefore, reference values established for cattle cannot be used for the clinical evaluation of neonatal calves. Studies presenting a set of clinical, haematological, and biochemical reference values for neonatal calves are required to support future projects and biotechnological solutions involving the health of newborn calves.

# Specifications Table



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Research article



#### Introduction

After calving, many changes occur in the newborn's body to adapt to extrauterine life. Several physiological functions develop during the neonatal period, including those of the cardiovascular, respiratory, metabolic, thermoregulatory, and immunological systems. Newborns are unstable, making them particularly sensitive to perinatal diseases, resulting in high mortality [\(Kasari, 1994;](#page-6-0) [Piccione et al., 2010](#page-6-0)). Even in farms with good management practices, high morbidity associated with diarrhoea and bovine respiratory diseases (BRDs), along with the highest neonatal mortality rate, is observed, incurring a significant economic loss ([Gomes](#page-6-0) [et al., 2021\)](#page-6-0).

Although several studies have been carried out with neonatal Holstein calves [\(Benesi et al., 2012a; Novo et al., 2015; Novo](#page-5-0) [et al., 2017; Baccili et al., 2018; Panousis et al., 2018\)](#page-5-0), most of them reported only haematological data. Few longitudinal studies have presented a set of physiological values in healthy neonatal Holstein calves including vital signs and biochemical parameters. Given the importance of neonatal physiological reference values, the aim of this study was to evaluate vital signs (rectal temperature and heart and respiratory rates) and to measure haematology and biochemical parameters (total protein, albumin, globulin, immunoglobulin G [IgG], haptoglobin, iron, glucose, non-esterified fatty acids [NEFAs], beta hydroxybutyrate [BHB], total cholesterol, and triglycerides) in 28 neonatal Holstein female calves in order to obtain valuable information on extrauterine adaptation. The

results of this could help field veterinarians identify diseases and may serve as a reference for future studies on newborn calves.

## Material and methods

#### Study area and climatic condition

This study was performed from July to October 2018 on a commercial dairy farm located in Descalvado, São Paulo, Brazil. During July and August, the average maximum, minimum, and thermal amplitude temperatures were 28.2  $\degree$ C, 9.2  $\degree$ C, and 21.2  $\degree$ C, respectively, while those in September and October were 29.3  $\degree$ C, 15.9  $\degree$ C, and 13.4  $\degree$ C, respectively.

#### Management of animals and experimental design

Twenty-eight young female Holstein heifers, calved of eutocia from healthy, multiparous Holstein dairy cows, between the 2nd and 5th lactation were screened. Cows were dried off at approximately 220 days of gestation and transferred to an indoor compost-barn system with wind tunnel ventilation 30 days before the expected calving date. Shortly after birth, calves were separated from their dams and placed in a ''cuddle box" to receive initial maternal contact and a ''sniff and lick" from their mothers. The dams were milked immediately after calving using a portable milking machine at the maternity pen. Only dams that produced a minimum volume of 3 L of colostrum with  $\geq$  21% BRIX or refractometry index were included in this investigation to avoid the failure of passive immune transfer. The first colostrum feeding was provided using a 3-L bottle offered within an hour after delivery on D1. Until D14, calves were housed in closed-sided individual housing with a bed of hay in a covered barn. During this period, each calf was fed 6 L of non-medicated milk replacer (Nattimilk E Max<sup>®</sup>, Auster Nutrição Animal Ltda, Brazil – Supplemental Table S1) divided into two feedings (0700 h and 1500 h). The dilution used to prepare the milk replacer was 1 kg/7 L of water, constituting 10% total solids. Milk was offered in bottles until D2–D3, and later in buckets fixed in each pen, restricted to every individual calf. The water was introduced on D2 and farm-produced starter without probiotics (Supplemental Table S1-S3) was introduced on D3; both were supplied until the end of the experiment, that is, the same concentrate (starter) was supplied in phase 1 and phase 2 of the experiment.

On D15, animals were transferred to another calf-rearing unit (phase 2) inside the same commercial farm and housed in a closed-sided individual housing with a hay bed. Until D28, they received 8 L of transitional milk pool supplemented with milk replacer, as two feedings per day, to correct the DM. During this phase, the animals received ad libitum feeding of a farmproduced starter; it was ensured that the diet met or exceeded the nutritional requirements of preweaned Holstein calves to achieve a maximum growth rate [\(NRC, 2001\)](#page-6-0). After D28, calves remained in the rearing unit used for the study [\(Santos, 2020](#page-6-0)).

# Sampling of blood

Blood samples of neonatal calves were obtained via jugular venipuncture immediately after birth and before colostrum intake on D1, and D2, D7, D14 and D28. The sampling time was 2 h before the morning milk feeding, when calves were physically examined to measure heart rate (HR), respiration rate (RR), and rectal temperature (RT). Faecal and BRD scores were assessed according to the Calf Heath Scoring Criteria of the University of Wisconsin ([Mcguirk, 2008\)](#page-6-0). Samples of animals that presented faecal scores of 2 or 3 on the day of collection or those satisfying a minimum of two parameters of the BRD assessment were characterised as calves with respiratory disease and thus were excluded. Only two animals presented BRD, one at D1 (Calf 18) and other (Calf 1) at D28, so 3% (1/28) at these time points. Two calves (calves 4 and 27) were excluded from all analysis times, as they were not healthy due to diarrhoea (faeces score 2 or 3), so the final sample number was 26 animals. At D28, six calves were not included in the analysis, as there was a technical problem with the sample and haematological equipment, which is why there is no leukocyte differential in the worksheet. Furthermore, at the same time, seven animals were disregarded because they had diarrhoea, with a score of 2 (n = 6) and a score of 3 (n = 1). Thus, on D28, 13 animals were not considered in the statistical analysis. As this study was designed to examine the developmental markers of healthy calves, animals that showed evidence of disease were eliminated.

Blood was collected from the calves into three plain vacuum tubes: two containing fluoride sodium, with and without an anticoagulant, to obtain plasma and serum, respectively, and another with ethylenediaminetetraacetic acid (EDTA) to perform blood count. Blood was allowed to clot at room temperature and serum/ plasma was subsequently obtained after centrifugation at 2 000  $\times$ g for 15 min, aliquoted, and stored at  $-20$  °C, ideally for 3 months after collection. EDTA samples were refrigerated at  $4 \degree C$  and analysed within 3 h of collection.

Blood was not collected on D1 since the calving times were different, and the laboratory processing was not performed at the farm; therefore, collections were grouped from D3 to D28, thus resulting in four samples (D3, D7, D14, and D28) for data analysis.

## Analysis of blood parameters

Biochemical biomarkers were measured using specific commercial kits for each parameter as specified by the manufacturer. Quantification of cholesterol (Labtest®, 76-2/100, Brazil), triglycerides (Labtest®, 87-2/250, Brazil), iron (Randox®, SI250, United Kingdom), total protein (Labtest®, MG3880, Brazil), and albumin (Labtest<sup>®</sup>, 19-1/250, Brazil) was performed from serum samples, while that of glucose (Labtest®, 133-1/500, Brazil), NEFA (Randox®, FA115, United Kingdom), and BHB (Randox®, RB1007, United Kingdom) were obtained from fluoride plasma samples. Aliquots were thawed overnight in a refrigerator at  $4^{\circ}$ C. When the samples were fully thawed, they were homogenised by vortexing, and biochemical tests were performed using an automated biochemical analyzer (Labmax 240, Labtest®, Japan).

Complete and differential blood counts were obtained using an automated haematology system (ADVIA 2120i; Siemens Healthcare Diagnostics, Tarrytown, NY, USA). The concentration of haptoglobin (Hp) was determined based on its ability to bind to haemoglobin (Hb), using spectrophotometry, as described by [Ramos et al. \(2021\).](#page-6-0) Serum IgG levels were quantified using an in-house sandwich ELISA according to the procedure described by [Reber et al. \(2006\)](#page-6-0).

#### Statistical analysis

All data assessments were performed using Statistical Analysis System for Windows (version 9.2, SAS Institute Inc., Cary, NC, USA) and were tested for residual normality and variance homogeneity. Variables without an endogenous normal distribution were forcefully transformed to fit the statistical assumption of normality, using log 10, square root, or inverse treatment of whole datasets. The outliers detected by command-guided data analysis were individually evaluated; they were included in the statistical analysis after the raw data were subjected to correction for typing errors. A PROC ANOVA was performed to determine the overall effect of age on each variable. The pairwise differences between the age clusters of calves were tested using parametric tests in the GLM package, allowing for individual assessment of each pair of factors. Differences among the three age clusters were analysed using Tukey's test and reported as untransformed mean ± SE. Statistical significance was set at  $P \leq 0.05$ .

# Results

The vital signs of the calves (HR, RR, and RT) during the neonatal period are shown in [Fig. 1.](#page-3-0) HR and RR were highest on D1, followed by a gradual decrease, with the lowest on D14, and returned to their original rates on D28. RT was lowest on D1.

The biochemical test results are presented in [Table 1.](#page-3-0) The concentrations of glucose, total proteins, globulins, and IgG were lowest on D1; they rose to the highest on D3, followed by a gradual decrease. Albumin concentration, on the other hand, was lower on D3 than on D1 but was later found to gradually increase to its peak on D28. Cholesterol gradually increased in concentration from D1 to reach its peak on D28, while a time-dependent difference was not observed for NEFA, BHB, triglycerides, iron, and Hp.

The results of the haematological analysis are presented in [Table 2.](#page-4-0) In addition to an increase in Cellular Haemoglobin Content (CHC), a decrease in haemoglobin, haematocrit, and mean corpuscular volume (MCV) was detected on D28. While the platelet count was lowest on D3, indistinguishable higher values were observed from D7 to D28. The total leukocyte count did not show variation; however, on D3, there was a greater number of circulating neutrophils, which decreased on D7 and increased again on D14 and D28. The lymphocyte count was lowest on D3; it reached its peak between D7 and D14 and reduced non-significantly on D28. The other variables did not show any time-dependent differences.

## Author's points of view

The calves in this study showed the highest HR and RR on D1 and decreased rates from D14. Similar results were found by [Lisbôa et al. \(2003\),](#page-6-0) who reported a decrease in HR and RR with advancing age, with the highest rate observed in the first week of life. [Egli and Blum \(1998\)](#page-6-0) observed the highest HR on D1, a decrease until D21, and an increase on D28, concurrent with our findings. [Silva et al. \(2016\)](#page-6-0) also observed a peak in HR and RR during the first evaluation period after birth, with a decrease in their values in the subsequent weeks.

A high HR soon after birth is explained by the fact that the neonate's heart needs to pump blood in a highly elastic vascular system, with more peripheral resistance; however, the systolic volume of the neonate's heart is small and limited, requiring a higher HR to achieve an adequate cardiac output. The heart rate decreases with age as the cardiovascular system adapts ([Piccione](#page-6-0) [et al., 2010; Silva et al., 2016\)](#page-6-0).

Transient tachypnea observed after eutocic deliveries is a result of umbilical vessel compression and reduced oxygen supply to the neonate. The highest RR in the first days of life may occur due to the diminished capacity of pulmonary respiratory function, owing to incomplete organ development observed at this stage [\(Feitosa](#page-6-0) [and Benesi, 2014](#page-6-0)). In this regard, one study reports that the lungs achieve their maximum gas exchange capacity on D14, confirmed by the partial pressures of oxygen and carbon dioxide [\(Linke](#page-6-0) [et al., 2013](#page-6-0)). RR variations in the first weeks of life reflect anatomical and functional changes, secondary to incomplete maturation and homeostasis of the respiratory system [\(Piccione et al., 2010;](#page-6-0) [Silva et al., 2016; Dantas et al., 2019\)](#page-6-0).

Neonates are more susceptible to environmental temperature variations, with a narrower body temperature regulation range than adults, accredited to their large body surface area, amniotic fluid evaporation, and limited caloric reserves [\(Dantas et al.,](#page-5-0)

<span id="page-3-0"></span>

Fig. 1. Mean ± SE of vital signs (heart rate, respiration rate, and rectal temperature) of healthy Holstein calves during first month of life (D1, D3, D7, D14, and D28). Means with different lowercase letters above differed statistically by the Tukey test ( $P \le 0.05$ ) among the sample times.

#### Table 1

Biochemical parameters of Holstein calves in the first month of life.



Abbreviations: RT = rectal temperature; NEFAs = non-esterified fatty acids; BHB = b-hydroxybutyrate; TP = total protein; Hp = haptoglobin.

Data presented as mean  $\pm$  SE. Means followed by the different lowercase letters in the column differed statistically by the Tukey test ( $P$  < 0.05).

[2019\)](#page-5-0). In the first hours after birth, calves have a lower RT due to greater heat loss and lower efficiency of thermal regulation; thus, their metabolic activity increases by using energy substrates, obtained from ingested colostrum and milk and reserves of glycogen and brown fat, which are converted into energy and heat to regulate body temperature [\(Feitosa and Benesi, 2014](#page-6-0)).

In a study by Lisboa et al. (2003), RT was low close to birth and increased by D2, remaining stable at the other evaluation timings. According to [Egli and Blum \(1998\)](#page-6-0), RT increased from D1 to D14, and in hypothermic animals, RT increased rapidly after the first feeding, later maintaining the pattern observed in other animals. This finding is consistent with the results of this study, showing greater susceptibility to heat loss on D1 and increased RT subsequently.

Colostrum contains proteins, fat, lactose, minerals, vitamins, both essential and non-essential amino acids, and fatty acids, as well as immunoglobulins, growth factors, hormones, and other components ([Mcgrath et al., 2016](#page-6-0)). Colostrum is essential for calves in the first hours of life because of its great nutritional and immunological value, reflective of the immunoglobulin absorption, referred to as passive immunisation ([Lopez and Heinrichs, 2022\)](#page-6-0). Colostrum has a large amount of energy, which is compensatory of the neonates' limited energy reserves, thus influencing the body temperature and heat production through the digestion, absorption, and metabolism of its nutrients ([Quigley and Drewry, 1998;](#page-6-0) [Novo et al., 2017](#page-6-0)).

After birth, the energy demands of the calf, previously met by placental transfer of glucose, are now met by colostrum and ingested milk, containing lactose and fat as the main energy sources; thus, though calves are born with low glycemic levels, blood glucose increases with its absorption from the lactose in colostrum ([Hammon et al., 2013, Egli and Blum, 1998\)](#page-6-0). However,

#### <span id="page-4-0"></span>Table 2

Haematological parameters of Holstein calves in the first month of life.



Abbreviations: RBC = red blood cell count; HBG = haemoglobin; HTC = haematocrit; MCV = mean corpuscular volume; CHC = cellular haemoglobin content; MCHC = mean corpuscular haemoglobin concentration; WBC = white blood cell count.

Data presented as mean  $\pm$  SE. Means followed by the different lowercase letters in the column differed statistically by the Tukey test ( $P$  < 0.05).

calves also depend on the endogenous production of glucose by gluconeogenesis and glycogenolysis; the highest glucose concentration on D2 is reflective of the maturation of the gluconeogenic capacity, in addition to the absorption of lactose from colostrum and milk ([Scheuer et al., 2006; Steinhoff-Wagner et al., 2011\)](#page-6-0).

It is argued that the increase in glucose in the first hours of life is due to colostrum intake and the higher concentration of corticosteroids following calving [\(Mohri et al., 2007\)](#page-6-0). Similar to the results of this study, [Egli and Blum \(1998\)](#page-6-0) found lower blood glucose levels on D1 than on D2. [Knowles et al. \(2000\) and Mohri et al.](#page-6-0) [\(2007\)](#page-6-0) also found an initial increase in blood glucose in the first days of life; however, a small reduction was observed in the following weeks. While [Knowles et al. \(2000\)](#page-6-0) observed a decrease from the second week onwards, [Mohri et al. \(2007\)](#page-6-0) reported a decrease on D14, which remained stable until D28; these corroborate our study findings. In the evaluated heifers, there was a progressive increase in serum cholesterol concentrations from D1 to D30. Cholesterol can be absorbed from the diet or synthesised by the liver, and it is intrinsic to the lipoproteins that transport lipids in the bloodstream [\(Bruss, 2008](#page-5-0)). The cholesterol in milk could contribute to its increase in serum during the first month of life; it is consistent with an earlier report on the dietary contribution ([Piccione et al., 2010\)](#page-6-0).

Neonates adapt nutritionally from a carbohydrate-based energy supply in the foetal period to a high-fat, relatively lowcarbohydrate supply in the colostrum ([Godden et al., 2019\)](#page-6-0). Colostrum ingestion on D1 plays a role in increasing the lipid profile ([Blum et al., 1997](#page-5-0)). Cholesterol is an indicator of energy metabolism, related to the transport of lipids in the form of lipoproteins ([Ndlovu et al., 2007\)](#page-6-0), and thus, lipid metabolism may have influenced the cholesterol increase found in this study.

The alterations found in the white blood cell parameters were similar to those reported by other authors; while soon after delivery, the number of neutrophils was greater than that of lymphocytes, the ratio reversed with time ([Knowles et al., 2000; Brun-](#page-6-0)[Hansen et al., 2006](#page-6-0)). Until D30, the highest and the lowest numbers of neutrophils and lymphocytes were found on D1, respectively, with an inversion of the neutrophil-lymphocyte ratio occurring on D4 due to a significant reduction in the neutrophil count within 3 days and an increase in lymphocytes in the second week of life ([Benesi et al., 2012b\)](#page-5-0).

Higher concentrations of glucocorticoids, including those of endogenous origin in the foetus, as well as those released in late pregnancy and during eutocia, lead to neutrophilia and lymphopenia ([Mohri et al., 2007; Benesi et al., 2012b; Baccili et al., 2018\)](#page-6-0). This explains the early leukogram changes, which are followed by a gradual reduction in the neutrophil count. The neutrophillymphocyte ratio inversion is called a stress leukogram and is physiologically observed until D4 [\(Baccili et al., 2018\)](#page-5-0).

A higher lymphocyte count was observed in the second week of life, corroborating the immunological maturation of calves ([Brun-](#page-5-0)[Hansen et al., 2006\)](#page-5-0). The increase in lymphocytes is attributed to the greater number of circulating B lymphocytes, indicating the development of the adaptive immune system and the consequent generation of an immune response [\(Baccili et al., 2018](#page-5-0)). [Benesi et al. \(2012b\)](#page-5-0) found a higher lymphocyte count between D21 and D25.

After colostrum ingestion and protein absorption, an osmotic effect of red blood cells (RBCs) results in haemodilution, reduction of haematocrit, haemoglobin, and erythrocyte values in the early days of life ([Egli and Blum, 1998; Rocha et al. 2013, Baccili et al.,](#page-6-0) [2018; Panousis et al., 2018\)](#page-6-0). However, in this study, erythrocyte evaluation was performed only from D3, and hence, this effect could not be assessed. The average haematocrit level was 33.6% on D3, similar to the results of other authors. [Baccili et al. \(2018\)](#page-5-0) reported 39% as the mean haematocrit level on D0; it fell to 31% on D2 and 30% on D4, similar to the report of [Rocha et al. \(2013\)](#page-6-0) on calves from primiparous cows, which reported values of 42% on D0, 37.8% on D1, and 34.6% on D7.

The erythrogenesis phase between D3 and D5, associated with an increase in the reticulocyte count [\(Benesi et al., 2012a\)](#page-5-0), could explain the slight increase in haematocrit and haemoglobin concentrations, observed on D7 and D14, when compared to those observed on D3. [Panousis et al. \(2018\)](#page-6-0) reported similar RBC parameters from D1 to D9 in Holstein calves.

Significant reduction was noted in the haematocrit, haemoglobin, MCV, and MCHC values on D28, similar to earlier reports ([Egli and Blum, 1998; Brun-Hansen et al., 2006; Benesi et al.,](#page-6-0) [2012a\)](#page-6-0). Foetal erythrocytes with foetal haemoglobin and a higher MCV circulate for a shorter duration; they are replaced at birth by smaller-sized erythrocytes with mature haemoglobin, justifying a low MCV. Thus, a greater number of erythrocytes would be required to maintain the haemoglobin concentration; however, a deficient renewal rate could explain the findings, reflecting the low haematocrit and haemoglobin levels in calves [\(Egli and](#page-6-0) [Blum, 1998; Brun-Hansen et al., 2006; Benesi et al., 2012a;](#page-6-0) [BaccilI et al., 2018; Wood and Quiroz-Rocha, 2010](#page-6-0)).

The platelet count is varyingly reported to increase in heifers from D7 [\(Egli and Blum, 1998; Khaleghnia et al., 2021\)](#page-6-0), D10 ([Knowles et al., 2000](#page-6-0)), or the second week of life [\(Brun-Hansen](#page-5-0) [et al., 2006](#page-5-0)); however, the reason for this increase could not be determined. [Khaleghnia et al. \(2021\)](#page-6-0) reported that parenteral administration of iron to newborn calves had no effect on erythro<span id="page-5-0"></span>poietic performance, thyroid metabolism, oxidative status, and health of dairy calves, with observations of blood parameters consistent with our study.

In cattle, the synepitheliochorial placenta blocks the transfer of immunoglobulins; thus, an agammaglobulinemic neonate, with an immature immune system that cannot produce sufficient antibodies, acquires immunoglobulins through the ingested colostrum ([Godden et al., 2019\)](#page-6-0). Therefore, albumin is the predominant plasma protein, in addition to globulins of hepatic origin, with minimal immunoglobulins [\(Wood and Quiroz-Rocha, 2010](#page-6-0)).

There is a significant increase in plasma protein concentration with colostrum ingestion [\(Knowles et al., 2000](#page-6-0)) with respect to immunoglobulins [\(Egli and Blum, 1998](#page-6-0)) and globulins ([Mohri](#page-6-0) [et al., 2007; Júnior et al., 2013](#page-6-0)) and cannot be attributed to that of albumin [\(Feitosa et al., 2001](#page-6-0)). The findings of increased IgG, globulins, and total protein with colostrum ingestion are consistent with previous reports ([Egli and Blum, 1998; Knowles et al.,](#page-6-0) [2000; Feitosa et al., 2001; Mohri et al., 2007; Júnior et al., 2013\)](#page-6-0); however, the concentrations fall with the time-dependent degradation of absorbed immunoglobulins [\(Feitosa et al., 2001; Mohri](#page-6-0) [et al., 2007\)](#page-6-0).

As for albumin, similar to what was found in this study, other authors reported an initial decrease in albumin concentration after colostrum ingestion due to the haemodilution effect following the absorption of other plasma proteins from colostrum ([Egli and](#page-6-0) [Blum, 1998\)](#page-6-0), contributing to an increase in the total body water ([Wood and Quiroz-Rocha, 2010\)](#page-6-0). However, after colostrum intake, albumin concentration gradually increases with time by hepatic synthesis and dietary absorption ([Egli and Blum, 1998; Feitosa](#page-6-0) [et al., 2001, Júnior et al., 2013\)](#page-6-0). Furthermore, [Mohri et al. \(2007\)](#page-6-0) suggested that this increase in albumin level may compensate for the decreased globulin-induced fall in serum osmotic pressure.

Haptoglobin, a positive acute-phase protein ([Ramos et al.,](#page-6-0) [2021\)](#page-6-0), did not change over time, possibly because animals with infectious diseases (diarrhoea and BRD) were excluded from the study. Similar changes were observed with ferritin, another acute-phase reactant ([Khaleghnia et al., 2021\)](#page-6-0).

In conclusion, physical, haematological, and biochemical changes observed during the first month of life in calves are linked to extrauterine adaptation. While the age-related reduction in HR and RR represents the maturation of the cardiovascular and respiratory systems, respectively, the increase in RT represents that of thermoregulatory mechanisms. Colostrum intake is reflected in several blood parameters, including immunoglobulin absorption, and alteration of the serum protein profile. In addition, changes in glucose and cholesterol concentrations are reflective of the metabolic capacity of calves. Leukocyte changes, such as an increased lymphocyte count, are associated with maturation of the immune system, in addition to a decrease in neutrophils, secondary to the influence of cortisol concentrations at parturition. Changes in the erythrogram can be attributed to the replacement of circulating foetal erythrocytes. Thus, it is evident that there are variations in the physiological parameters of calves after birth, and for better clinical interpretation, it is necessary to compare these parameters with established age-specific reference values.

#### Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.anopes.2022.100036>.

# Ethics approval

All procedures involving animals were approved by the Animal Research Ethics Committee of the School of Veterinary Medicine

and Animal Science, at the University of São Paulo (protocol number 6740260218).

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# Author contributions

FCRS: Data Curation, Formal analysis, Investigation, Methodology. BPS, FE DM, ECBPG: Writing - Original draft, Writing - Reviewing and Editing. KNS: Methodology. VG: Visualisation, Supervision, Writing - Reviewing and Editing.

# Declaration of interest

None.

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