Hematological adaptation in Holstein calves during the neonatal period

Adaptação hematológica de bezerras Holandesas durante o período neonatal

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Abstract

The aim of this study was to evaluate the hematological profile of Holstein calves during the first month of life. Blood samples were harvested (n = 208) from 26 calves, from birth until 30 days of life. Hematologic values were determined by an automatic system associated with differential leukocyte count by manual methods. Variations in the erythrogram components were detected from birth up to the 30th day of life, except for hemoglobin (Hb) concentration and Mean Corpuscular Hemoglobin Concentration (MCHC). At birth, higher values were observed for hematocrit, Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) that decreased in subsequent moments. During the first days of life, leukocytosis was found due to neutrophilia and eosinopenia. A gradual increase of lymphocytes with the increase of age was also observed. Finally, the present research showed that the first month of life is a hematological adaptation period. Based on the results, it detected that blood component variations, characterized by hemoconcentration and leukocyte prolife compatible with glucocorticoids response up to the 4th day of life, were responsible for neutrophil lymphocyte ratio > 1.0 at birth.

Keywords: Cattle. Newborns. Blood count. Neutrophil-lymphocyte ratio.

Resumo

Foi avaliado o perfil hematológico de bezerros da raça Holandesa durante o primeiro mês de vida. Foram colhidas 208 amostras de sangue total de 26 bezerras(os) do nascimento aos trinta dias de vida. Os valores hematológicos foram determinados por sistema automatizado associado à contagem diferencial dos leucócitos por metodologia manual. Foram detectadas variações nos componentes do hemograma do nascimento aos 30 dias de vida, exceto para os teores de hemoglobina (Hb) e concentração hemoglobínica corpuscular média (CHCM). Os maiores valores do hematócrito, volume corpuscular médio (VCM) e hemoglobina corpuscular média (HCM) foram observados ao nascimento, com decréscimo nos momentos subsequentes. Nos primeiros dias de vida foi observada leucocitose por neutrofilia e eosinopenia e com o avançar da idade houve aumento gradativo dos linfócitos. Com base nos resultados obtidos pode-se concluir que a adaptação dos bezerros no período pós-neonatal foi caracterizada por variações nos componentes do hemograma, observando-se hemoconcentração e padrão leucocitário compatível com resposta aos glicocorticoides até o 4º dia de vida, responsável pela relação neutrófilo-linfócito > 1,0 ao nascimento.

Palavras-chave: Bovinos. Recém-nascidos. Hemograma. Relação neutrófilo-linfócito.

Introduction

The neonatal period of calves is characterized by an increase of disease susceptibility, especially diarrhea, umbilical cord inflammation and bronchopneumonia due to immunological immaturity and agammaglobulinemic status of newborns at birth. During this period, they are dependent on maternal immunity transference by colostrum intake (CHASE; HURLEY; REBER, 2008).

To reach the goals of morbidity under 5% and mortality under 10% in the raising of heifers at birth up to weaning (PRODUCTION, 2013) requires health monitoring that includes physical and laboratory examination for early diagnosis and treatment. The automation of laboratory processes allows rapid

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analysis of blood components and enables the use of this method for calf disease diagnosis (KNOWLES et al., 2000). However, data interpretation is dependent on knowledge of physiology and reference intervals compatible with each species, breed, age and geographical position (KNOWLES et al., 2000; MOHRI; SHARIFI; EIDI, 2007).

Changes are observed in the hematological profile of calves due to the transition from the intrauterine environment to the external environment (KNOWLES et al., 2000; BENESI et al., 2012a). Influences of age on reference intervals for blood components have been reported in calves; however, longitudinal studies with repeated measurements are scarce (BRUN-HANSEN; KAMPEN; LUND, 2006).

The study of hematological profile of healthy newborn calves during the organic adaptation period to the environmental challenge is fundamental. Thus, the aim of this study was to perform a longitudinal study to evaluate the hematological profile of Holstein calves during the postnatal period.

Materials and Methods

Male and female Holstein calves were used for this research. Births were monitored for newborn vitality evaluation by modified APGAR test (BORN, 1981), umbilical cord disinfection and colostrum intake. Neonates were transferred to stalls and suckled with six liters of replacer milk, divided in two daily feedings. Water and starter feed were offered ad libitum to accelerate the development of pre-stomachs.

Whole blood samples (n = 208) from 26 neonates were obtained at the following time: up to six hours after birth (a.b.) - T0; 1-2 days a.b. - T1; 3-4 days a.b. - T2; 5-6 days a.b. - T3; 7-8 a.b. - T4; 9-10 days a.b. - T5; 15 days a.b. - T6; and 30 days a.b. - T7. After that, the external jugular vein was punctured using a vacuum system and tubes containing ethylenediaminetetraacetic acid (1.5mg/mL). Samples were sent to the laboratory under refrigeration (4°C).

Erythrogram and total leukocytes were analyzed by automatic counting (ABC Vet, ABX®). Differential count was performed by leukocyte differentiation using an optical microscope with 1000x magnification.

Statistical analysis was developed using the software SAS version 9.3 for Windows (SAS Institute Inc, Cary, NC,USA). Blood parameters presented normal distribution according to the Kolmogorov-Smirnov test. Variance analysis was performed by ANOVA test for repeated measures in times and Tukey's test for comparison of means. Differences were considered significant when $P < 0.05$.

Results and Discussion

This research evaluated the hematological profile of healthy newborn calves from birth up to 30 days of life, a period marked by organic adaptations to environment and immunological immaturity.

According to data presented in Tables 1 and 2, the results obtained in this study reveal that there were variations in the components of the erythrogram and leukogram of neonates during the first month of life.

Erythrogram analysis showed hemoconcentration at birth, characterized by maximum mean value of Ht. It was possible to observe a decrease of these values up to 10 days of life and an increase between T6 and T7. The maximum peak of Hb was also observed in T0, and followed by a decrease and stabilization in subsequent moments. Similar variations have been reported previously in a longitudinal study by Knowles et al. (2000) and Mohri, Sharifi and Eidi (2007); however, data found in this research were different from those presented by Benesi et al. (2012a) who observed no changes in erythrogram components (He, Hb and PCV) in calves from birth up to 30 days of life. The divergence between researches may have occurred due to the adoption of different research models, considering that horizontal studies do not exclude individual variations (n = 300) and could explain the absence of changes in Benesi et al. (2012a).

Lower concentrations of oxygen in the placental environment are compatible with high concentrations of Hb at birth. Other hypotheses related to erythrogram component decrease has been associated with higher serum protein concentration after colostrum intake, which increase oncotic pressure and dilute plasma (SCHALM, 2010); or with low serum iron concentrations in the newborn, impacting in erythropoiesis and, thus, decreasing the values of He, Ht and Hb in the first month of life (MOHRI et al., 2004; MOHRI; SHARIFI; EIDI, 2007).

Oscillations for MCV and MCH were detected by a gradual decrease of their values at birth up to 30 days of life. Such events coincide with the replacement of fetal Hb to adult Hb. The decrease in Ht and MCV in the first month of life has been interpreted as transient microcytic anemia, considering the reference ranges for adult cattle (MOHRI et al., 2004; MOHRI; SHARIFI; EIDI, 2007).

The decrease of VCM values in the first month of life can be compensated by a phenomenon observed in this study during the 30 days of life: an increased number of red blood cells (BRUN-HANSEN; KAMPEN; LUND, 2006).

This study obtained maximum value of total leukocytes (14x10³/µl) and neutrophils (8,5x10³/µl) during the first hours after birth, followed by a gradual decrease from T1 (48 hours old) to T7 (30 days of life). The reduction in postnatal cells was more intense for neutrophils (Ne).

Lymphocytes and monocytes showed minimum values in the first two days of life, followed by gradual increase, with maximum values observed at 30 days of life - T7 (Lymphocytes = 6,9 x10³/µl; Monocytes = 2,1 x10³/µl). Eosinophils also showed increasing values from birth up to T6, and basophils were not found during the studied period.

Table 1 – Erythrogram constituent values (mean ± SD) of Holstein calves at birth up to 30 days of life – São Paulo – 2011-2013

Moments	RBC (x10⁶/µL)	Hb (g/dL)	Ht (%)	MCV (fl)	MCH (pg)	MCHC (%)
T0 (<6 hours)	$8.3^{b} \pm 1.4$	$11.5^{a} \pm 2.5$	$37.3^{a} \pm 7.2$	$45.4^{a} \pm 3.6$	$13.8^{a} \pm 1.6$	$30.5^{a} \pm 2.4$
T1 (1 - 2d)	$7.1^{b} \pm 1.2$	$9.8^{a} \pm 1.9$	$30.2^{b} \pm 6.0$	$42.6^{b} \pm 3.0$	$13.8^{a} \pm 1.2$	$32.4^{a} \pm 2.2$
T2 (3 - 4d)	$7.2^{b} \pm 1.2$	$9.7^{a} \pm 2.4$	$30.1^{b} \pm 5.9$	$41.5^{bc} \pm 2.8$	$13.5^{a} \pm 1.5$	$32.5^{a} \pm 3.0$
T3 (5 - 6d)	$7.2^{b} \pm 1.4$	$9.8^{a} \pm 1.8$	$30.0^{b} \pm 5.5$	$41.2^{bc} \pm 2.4$	$13.0^{a} \pm 2.2$	$31.6^{a} \pm 4.6$
T4 (7 - 8d)	$7.5^{b} \pm 1.6$	$10.1^{a} \pm 1.8$	$31.5^{b} \pm 5.7$	$41.4^{bc} \pm 2.2$	$13.0^{ab} \pm 1.3$	$31.6^{a} \pm 3.1$
T5 (9 - 10d)	$7.7^{b} \pm 1.3$	$10.1^{a} \pm 1.6$	$31.8^{b} \pm 4.8$	$41.2^{bc} \pm 2.0$	$13.0^{ab} \pm 1.0$	$31.7^{a} \pm 2.3$
T6 (15d)	$8.2^{b} \pm 1.3$	$10.4^{a} \pm 1.5$	$33.0^{ab} \pm 5.2$	$39.7^{c} \pm 2.7$	$13.0^{a} \pm 3.6$	$32.6^{a} \pm 9.5$
T7 (30d)	$9.7^{a} \pm 1.9$	$10.9^{a} \pm 1.9$	$35.1^{ab} \pm 7.2$	$36.4^{d} \pm 1.6$	$11.3^{b} \pm 0.8$	$31.0^{a} \pm 2.0$

Different lowercase letters in the same row indicate statistical differences
RBC: red blood cells; Ht: hematocrit; Hb: hemoglobin; MCV: mean corpuscular volume; MCHC: mean corpuscular hemoglobin concentration; MCH: mean corpuscular hemoglobin; d: days after birth

Table 2 - Leukogram constituent values (mean ± SD) of Holstein calves at birth up to 30 days of life – São Paulo – 2011-2013

Moments	Le (x10³/µL)	Ne (x10³/µL)	Ly (x10³/µL)	Mon (x10³/µL)	Eos (x10³/µL)	Ne:Ly
T0 (<6 hours)	$14.4^{a} \pm 4.7$	$8.5^{a} \pm 3.8$	$4.6^{b} \pm 2.5$	$1.3^{b} \pm 0.6$	$0.0^{b} \pm 0.0$	$2.6^{a} \pm 2.4$
T1 (1 a 2d)	$12.9^{ab} \pm 4.6$	$6.9^{ab} \pm 3.6$	$4.5^{b} \pm 1.7$	$1.4^{b} \pm 0.9$	$0.1^{ab} \pm 0.1$	$1.7^{ab} \pm 1.1$
T2 (3 a 4d)	$10.6^{b} \pm 5.4$	$4.0^{c} \pm 3.7$	$4.8^{b} \pm 2.3$	$1.6^{ab} \pm 0.6$	$0.1^{ab} \pm 0.1$	$0.9^{bc} \pm 0.6$
T3 (5 a 6d)	$10.8^{ab} \pm 4.9$	$2.8^{c} \pm 1.5$	$5.4^{ab} \pm 2.4$	$1.9^{ab} \pm 0.9$	$0.1^{ab} \pm 0.2$	0.7c± 0.8
T4 (7 a 8d)	$10.6^{b} \pm 3.2$	$3.7^{c} \pm 2.2$	$5.0^{ab} \pm 1.8$	$1.6^{ab} \pm 0.6$	$0.1^{ab} \pm 0.1$	$0.9^{bc} \pm 1.0$
T5 (9 a 10d)	$11.6^{ab} \pm 3.4$	$4.5^{bc} \pm 2.5$	$5.5^{ab} \pm 1.9$	$1.5^{ab} \pm 0.7$	$0.1^{ab} \pm 0.1$	$0.9^{bc} \pm 0.6$
T6 (15d)	$11.0^{ab} \pm 2.9$	$3.3^{c} \pm 2.2$	$5.6^{ab} \pm 1.8$	$1.8^{ab} \pm 0.6$	$0.2^{a} \pm 0.2$	$1.2^{bc} \pm 1.9$
T7 (30d)	$11.7^{ab} \pm 3.3$	$2.5^{c} \pm 1.8$	$6.9^{a} \pm 2.5$	$2.1^{a} \pm 0.8$	$0.2^{a} \pm 0.2$	$1.0^{bc} \pm 2.3$

Different lowercase letters in the same row indicates statistical differences
Le: leukocytes; Ne: neutrophils; Ly: lymphocytes, Mon: monocytes; Eos: eosinophils; Ne:Ly: neutrophils:lymphocytes ratio

The leukocytosis by neutrophilia and eosinopenia observed in the first days of life may have occurred due to the release of maternal and fetal cortisol in late pregnancy and delivery. This fact resulted in neutrophils-lymphocytes ratio above 1 during the first 48 hours of life (Figure 1). It was observed a decrease of the ratio in the subsequent moments due to lower values of Ne. The neutrophil predominance at birth was also reported by Benesi et al. (2012b).

Cortisol stimulates the release of segmented neutrophils and band cells from the bone marrow compartment to circulation. In addition, cortisol influences neutrophil migration by reducing the expression of adhesion molecules and decreasing the connection of these cells to endothelial cells, which results in neutrophilia, with a marginal compartment reduction. Other hypotheses that could explain the neutrophilia would be the stimulation of stem cells for proliferation and differentiation of myeloid cells by the increase of colony-stimulating factor of granulocytes (SCHALM, 2010).

Red blood cell variations in neonatal calves are probably due to the availability of environmental oxygen and the iron metabolism. Leukogram variations were derived from the stress that starts at the end of pregnancy and parturition, followed by the environment adaptation challange. Findings of this research demonstrate that hemogram interpretation of neonates with diarrhea, bronchopneumonia, umbilical cord infection, among others, requires the adoption of reference intervals consistent with age, being unfeasible to use established values for adult animals (BRUN-HANSEN; KAMPEN; LUND, 2006).

Conclusions

We concluded that the adaptation of calves in the postnatal period was characterized by variations in the hematological profile. Hemoconcentration and leukocyte profile compatible with the glucocorticoids response, from birth up to 4th day of life, was found to be responsible for neutrophil lymphocyte ratio > 1.0.

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Ethics Committee and Biosafety

This study was approved by the Ethics Committee on Animal Use FMVZ-USP (Process n. 38/FMVZ/2012).

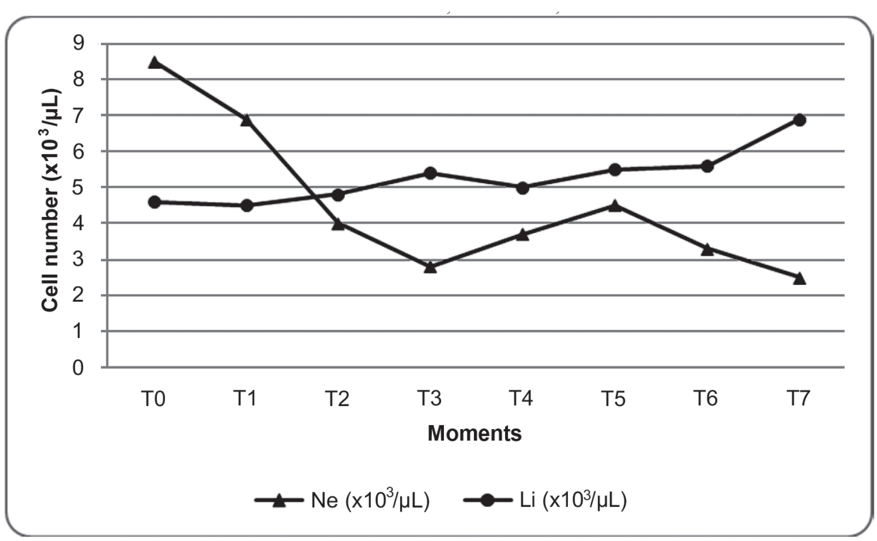

Figure 1 – Absolute values for neutrophils and lymphocytes ($x10^3/\mu L$) in blood samples from Holstein calves in the first month of life, Sao Paulo, 2011-2013
Ne: neutrophils; Li; lymphocytes; T0: <6 hours of life; T1: 1 – 2 days (d); T2: 3 - 4d; T3: 5 - 6d; T4: 7 - 8d; T5: 9 - 10d; T6: 15d; T7: 30d of life

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