

Original Research Paper

Evaluation of Some Physical, Haemathological and Clinical Chemistry Parameters in Healthy Newborn Italian Holstein Calves

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Abstract: The aim of the present study was to investigate some physical, haematological and clinical chemistry parameters in the newborn Italian Holstein calf at birth and at 24 h of life, to evaluate changes during the immediate post-partum period. Forty-six Italian Holstein Friesian calves were included in this study. Heart rate, respiratory rate and body temperature were recorded at birth and at 24 h of life. The time needed to raise the head, acquire sternal recumbency, stand up were also recorded. Blood samples were collected before first feeding and at 24 h of age and CBC count, L-lactate, glucose and total protein concentrations were evaluated. The head was raised immediately in 46/46 calves, suckling reflex was acquired within 12±9 min, sternal position in 5±2 min and newborn stood up in 38±30 min. Some of the physical data, haematological and biochemical values showed statistical differences between birth and 24 h of age. The results from this study provide some information about physical and laboratory data of Italian Holstein Friesian calves, at birth and at 24 h of life. Our results confirm that several clinical and laboratory values in newborn calves differ from adult reference intervals and from calves of different breeds.

Keywords: Calf, Neonatology, Hematology, Clinical Chemistry, Physical Data

Introduction

Newborn diseases and perinatal mortality are the major causes of economic loss in livestock production, especially in dairy cows (Mohri *et al.*, 2007; Mee, 2008). Perinatal mortality may be defined as calf death prior to, during or within 48 h of life, following a gestation period of at least 260 days (Mee, 2008). Management of the newborn dairy calf and a rapid diagnosis could limit economic loss and improve global farm health. The ability to interpret laboratory and clinical data is based on knowledge regarding the mechanisms underlying each alteration of normal physiologic values. Moreover, to better understand these data, a veterinarian needs reference values specific for each animal species and for age. Reference intervals commonly used for cattle are based on samples obtained from adult animals and they can be misleading if applied to young animals (Knowles *et al.*, 2000; Brun-Hansen *et al.*, 2006). The aim of the present study

was to investigate some physical and clinical chemistry parameters in the newborn Italian Holstein calf at birth and at 24 h of life to evaluate changes during this period.

Materials and Methods

Forty-six Italian Holstein Friesian calves were included in this prospective study. The 46 calves were born from 44 cows by vaginal delivery at the Pisa University dairy farm (Centro Interdipartimentale di Ricerche Agro-Alimentari "Enrico Avanzi", in San Piero a Grado-Pisa, Italy) and at the University Dairy Farm Unit of Bologna University. The farms were under health monitoring by the Veterinary school of Pisa and Bologna. Immediately after birth, each calf was separated from its dam and placed in an individual box with straw until the age of 14 days. All calves were fed with 2 L of dam's colostrums within the first 4 h of life and then twice a day with an amount of herd milk equal to 10% of their body weight via bucket feeding. Calves

had also free access to water. Inclusion criteria for cows were: physiological gestational length (>260 days) (Mee, 2008); transfer to the maternity unit at least 1 week before calving (Mee, 2008); Body Condition Score (BCS) of 3-3.5/5 (Stöber, 1993); normal parturition, without any manual, pharmacological or surgical assistance. Calves included in the present study followed these criteria: APGAR score ≥ 7 within 10 min from birth (Palmer, 2014); no clinical symptoms revealed by clinical examination performed at birth and at 24 h of life. The research protocol was approved by the Ethical Committee of the University.

Blood samples were collected from the jugular vein using a sterile 4 ml syringe within 15 min from birth (T0) before the assumption of colostrum and at 24 h of age (T24). Each blood sample was divided in two aliquots: 1 ml was collected in K3EDTA tube and 3 ml were stored in lithium-heparinized tube. The lithium-heparinized specimens were centrifuged at 3000 RPM for 10 min within 20 min from sampling, placed into pre-labeled sterile recipients and stored at -20°C until assessment. Plasma samples were analyzed in a single batch. Blood samples containing clots or grossly hemolysed were not processed.

The K3EDTA samples were processed 5 to 30 min after collection, in order to allow a full contact and interaction between blood cells and anticoagulant, to perform a Complete Blood Cell Count (CBC) with an automatic cell counting tool (Hemovet, SEAC, FI). Moreover, K3EDTA specimens were used to set up a blood smear, which has been air-dried and stained by an automatic stainer (Aerospray 7150 Hematology Slide Stain-Cytocentrifuge, USA). The differential cell count was performed by microscopic examination at 400 X and 1000 X magnification counting 100 cells. The K3EDTA samples collected at 24 h of life were processed in the same way. The differential count was used to calculate Neutrophil-Lymphocyte ratio (N/L) in order to verify the maturity of calves, as previously reported in foals (Axon and Palmer, 2008). Calves with $N/L > 2$ were considered mature.

Two 10- μL aliquots of heparinized-whole blood were analyzed immediately, one for glucose concentration and the other for L-lactate concentration using the Accu-Check Active[®] (Micralab srl, MI) and the Accutrend Lactate[®] (Micralab srl, MI), respectively, according to the instructions of the test kit manufacturer. Hand-holder meters

were previously validated for L-lactate (Baldari *et al.*, 2009; Karapinar *et al.*, 2013) and glucose (Katsoulos *et al.*, 2011). Plasma samples were used to quantify Total Protein (TP) concentration with a quantitative colorimetric method (Assel Srl, Rome, Italy).

For all the 46 newborn calves, the following data were recorded immediately after birth: (1) time to rise the head, (2) time to assume sternal position, (3) time to suckling reflex (stimulated by placing a finger in the mouth once), (4) time to stand. Furthermore, Heart Rate (HR), Respiratory Rate (RR) and Body Temperature (BT) were recorded both at T0 and T24.

Average (X), Standard Deviation (SD), Minimum (m) and Maximum (M) values were calculated for haematological, biochemical and physical results. The data were checked for normality using the Kolmogorov-Smirnov test. T test for paired data was performed if data distribution was Gaussian (PCV and PLT), while the Wilcoxon matched-pairs signed rank test was carried out on data with a non-Gaussian distribution (blood glucose, blood L-lactate, Hgb, WBC, RBC, MCV, MCH, MCHC and TP). Significance level was set at $p < 0.05$. All statistical analyses were performed using commercial software (Graph Pad Prism[®], USA).

Results

All the subjects raised the head immediately after birth and showed a suckling reflex within 12 ± 9 min (2-30 min). The time to assume sternal position was 5 ± 2 min, with a minimum value of 1 min and a maximum value of 15 min. The time to stand up was 38 ± 30 min, with a minimum value of 10 and a maximum of 105 min. The data for HR, RR and BT, both at birth and at 24 h of life, are shown in Table 1. None of the blood samples contained clots or was grossly haemolysed. CBC results at T0 and T24 are reported in Table 2. All the calves were considered mature on the basis of N/L ratio. Blood glucose, blood L-lactate and TP at birth and at 24 h of life are reported in Table 3. Significant differences between T0 and T24 values have been obtained for blood glucose, blood L-lactate, TP, RBC, PCV, Hgb and MCV ($p < 0.05$), while no differences have been found for the other evaluated parameters.

Table 1. Heart Rate (HR), Respiratory Rate (RR) and Body Temperature (BT) observed in a population of 46 healthy Italian Holstein Friesian calves at birth (T0) and at 24 h of life (T24). Legend - HR, bpm: Heart rate, beats/min; RR, bpm: Respiratory rate, breath/min; BT: body temperature; X \pm SD: Average \pm standard deviation; m: Minimum value; M: Maximum value. Within rows, * if $T0 \neq T24$ ($p < 0.05$).

HR (bpm)	RR (bpm)		BT ($^{\circ}\text{C}$)			
	T0	T24	T0	T24	T24	
X \pm DS	94.9 \pm 20.2	102.1 \pm 32.7	74.4 \pm 25.2*	51.6 \pm 17*	38.7 \pm 0.5	38.5 \pm 0.3
m	60.0	56.0	36.0	28.0	37.8	37.7
M	156.0	172.0	100.0	96.0	39.8	39.4

Table 2. Complete Blood Count (CBC) results at birth (T0) and at 24 h of life (T24) observed in a population of 46 healthy Italian Holstein Friesian calves. Legend - X±SD: Average ± standard deviation; m: Minimum value; M: Maximum value. Within rows, * if T0 ≠ T24 (p<0.05).

	WBC (K/ μ L)		RBC (M/ μ L)		HGB (g/dL)		PCV (%)		MCV (fL)		MCH (Pg)		MCHC (g/dL)		PLT (K/ μ L)	
	T0	T24	T0	T24	T0	T24	T0	T24	T0	T24	T0	T24	T0	T24	T0	T24
X±SD	10.7±3.8	9.0±3.1	8.1±1.3*	7.6±1.3*	10.7±1.8*	9.6±1.8*	36.5±7.3*	30.1±7.2*	44.3±4.7*	41.7±4.3*	12.9±1.0	12.5±1.0	28.8±2.6	29.7±1.7	328.0±100.0	290.6±93.0
m	5.7	4.5	5.0	5.5	7.7	5.7	27.2	17.9	34.0	32.6	11.0	11.0	22.4	25.4	168.0	124.0
M	21.9	18.5	11.1	10.4	14.7	12.5	57.0	47.3	54.1	53.0	15.6	15.6	34.9	34.9	558.0	511.0

Table 3. Blood glucose, L-lactate and total protein concentrations observed in a population of 46 healthy Italian Holstein Friesian calves at birth (T0) and at 24 h of life (T24). Legend - X±SD: Average±standard deviation; m: Minimum value; M: Maximum value. Within rows, * if T0 ≠ T24 (p<0.05).

	Glucose (mg/dL)		L-lactate (mmol/L)		TP (gr/dL)	
	T0	T24	T0	T24	T0	T24
X±DS	82±32*	133.1±31.4*	4.8±1.8*	3.7±1.3*	4±1*	5±1*
m	26	69.0	1.7	1.9	3	4
M	172	227.0	9.4	7.2	6	6

Discussion

In this study some physical and clinical parameters were investigated in newborn Italian Holstein calves during the first hours of life. Time to assume sternal position was in line with data reported by other authors (Mee, 2008; House and Vaala, 2009; Probo *et al.*, 2011). Time to stand up was slightly lower than that previously reported by Mee (2008; House and Vaala, 2009), but in line with others (Probo *et al.*, 2011).

Our findings on HR at birth and at T24 resulted lower than those previously reported in calves (Stöber, 1993; Mee, 2008; House and Vaala, 2009; Probo *et al.*, 2011). This could be related to differences in calves' management during physical examination. The average value of RR at T0 and T24, registered in this study, was similar to what reported in a previous study on newborn calves (Mee, 2008), while BT was lower than that reported by some authors (Mee, 2008; Probo *et al.*, 2011) and higher if compared to others (House and Vaala, 2009). If compared with adult reference ranges (Stöber, 1993), HR and RR resulted higher in calves than in adult, both at T0 and T24, while BT was similar.

WBC values at T0 and T24 were similar to those previously reported in Holstein and different breeds calves (Brun-Hansen *et al.*, 2006; Mohri *et al.*, 2007; House and Vaala, 2009; Probo *et al.*, 2011) and in adults (Radostits *et al.*, 1994). In our study, no statistical difference was found in WBC between T0 and T24. On these findings, WBC should not be influenced by colostrum ingestion and secondary hemodilution.

RBC values, registered in the present study, were in line with those reported by others, both at T0 (Knowles *et al.*, 2000; Brun-Hansens *et al.*, 2006; House and Vaala, 2009) and T24 (Mohri *et al.*, 2007; House and Vaala, 2009), but higher if compared with Probo *et al.* (2011). Recorded Hgb values were similar to previous reports, both at T0 and T24 (House and Vaala, 2009; Probo *et al.*, 2011), although they were slightly higher

at T24 if compared to those reported by Mohri *et al.* (2007). In our study, RBC and Hgb values, both at T0 and T24, were higher than those reported in adult animals (Radostits *et al.*, 1994; Wood and Quiroz-Rocha, 2010), except for a recent study, in which values were similar to ours (Morris, 2009). PCV values observed in this study were in line with those showed in calves by others, both at T0 and T24 (Knowles *et al.*, 2000; Mohri *et al.*, 2007; House and Vaala, 2009; Probo *et al.*, 2011). Our PCV results at birth were slightly higher than those reported for adult animals (Radostits *et al.*, 1994; Wood and Quiroz-Rocha, 2010); instead, our data at 24 h of life were similar to adult ranges (Radostits *et al.*, 1994; Smith, 2009; Wood and Quiroz-Rocha, 2010).

In the present study, RBC, Hgb and PCV values decreased significantly from birth to 24 h of age, in agreement with what reported earlier in newborn calves (Knowles *et al.*, 2000; Mohri *et al.*, 2007; House and Vaala, 2009). This may be related to hemodilution (although in this case it seems not to affect WBC), physiological destruction of erythrocytes by the spleen and/or decreased erythropoietin production secondary to increased blood oxygenation by lungs after birth, as already reported in foals (House and Vaala, 2009).

MCV, MCH and MCHC at T0 were similar to values reported by some authors (Knowles *et al.*, 2000; House and Vaala, 2009), while at T24 MCH resulted slightly higher than what found by Mohri *et al.* (2007); MCV was similar to and MCHC was lower than the findings reported by House and Vaala (2009). These differences could be related to different sampling time (within 24 h Vs 24 to 48 h or more). Statistical differences were found between sampling time for MCV, while no differences were observed for MCH and MCHC. It can be hypothesized that the decrease in MCV may be related to a physiological fetal erythrocyte disruption/elimination from the bloodstream and an increase in microcytes production, as occurring in foals (Harvey *et al.*, 1984; 1987; Axon and Palmer, 2008).

Comparing with Morris (2009), our results about MCV, MCH, and MCHC, both at birth and at 24 hours of age, were similar to those reported for adult animals. Instead, comparing with other authors (Radostits *et al.*, 1994; Wood and Quiroz-Rocha, 2010), we found the MCH concentration at birth slightly higher than adult ranges, but similar to adult animals at 24 h of life.

PLT at T0 was lower than previously reported (Knowles *et al.*, 2000), while at T24 our values resulted higher compared to Mohri *et al.* (2007), or still lower than Knowles *et al.* (2000). These differences could be due to different age (1 to 83 days) (Knowles *et al.*, 2000; Mohri *et al.*, 2007) or different breed. No differences were found between sampling times, but values, both at T0 and T24, were higher than those reported in adult animals (Radostits *et al.*, 1994; Wood and Quiroz-Rocha, 2010).

Blood glucose concentration, both at T0 and T24, was slightly higher than that reported by others in Holstein calves and calves of different breed (Knowles *et al.*, 2000; Mohri *et al.*, 2007), while TP concentration was similar (Knowles *et al.*, 2000; House and Vaala, 2009). Moreover, the glucose and TP mean values increased significantly from birth to 24 h of age and this could be explained by the colostrum intake (Knowles *et al.*, 2000). In the present study, blood glucose was always above adult reference ranges, while TP concentration was lower at T0 than in adults, but similar at T24 (Carlson, 2009). Our glucose and TP results were in line with data previously reported for calves at same age but different breeds (Knowles *et al.*, 2000). Blood L-lactate at T0 and T24 was similar to what previously reported and decreased significantly from birth to 24 h of life, when the adaptation to extra-uterine life should be completed (Szenci, 2012).

Data registered in the present study provide some information about physical and laboratory data of Italian Holstein Friesian calves, at birth and at 24 h of life and confirm that several parameters in newborn calves differ from adult reference intervals. A higher number of animals should be investigated, but these age and breed-related ranges can be useful during the evaluation of both healthy and sick newborn calves.

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Author Contributions

Francesca Bonelli: Study conception and design, analysis and interpretation of data, manuscript writing and reviewing, final approval.

Carolina Castagnetti: Study conception and design, analysis and interpretation of data, manuscript reviewing and final approval.

Eleonora Iacono: Study conception and design, data collection, analysis and interpretation of data, manuscript reviewing and final approval.

Michele Corazza: Study conception and design, manuscript reviewing and final approval.

Micaela Sgorbini: Study conception and design, analysis and interpretation of data, manuscript writing and reviewing, final approval.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

Conflict of Interest

None of the authors has any conflict of interest to declare.

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