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## Serum biochemical profile in Holstein Friesian and Belgian blue calves in the first 48 hours of life

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### ABSTRACT

Specific age-related changes in blood variables of calves have previously been reported. The very first hours after birth are however not fully investigated, and results originating from different breeds are combined. The purpose of this study was to investigate the variation in biochemical variables during the first 48 hours after birth in Holstein-Friesian (HF) and Belgian Blue (BB) calves. Nineteen HF calves born vaginally and 23 BB calves delivered by caesarean section were sampled within 30 min after birth, and at 24 and 48 h of life. The concentration of albumin, chloride, sodium, potassium, calcium, phosphate, urea, creatinine, glucose,  $\beta$ -hydroxybutyrate, total protein, and activity of AST,  $\gamma$ GT and glutamate dehydrogenase were evaluated. In both groups, significant decreases were recorded at 24 and/or 48 hours compared with 30 min for albumin, calcium, chloride and creatinine, while significant increases were found for AST,  $\gamma$ GT, bilirubin, GLDH, glucose and total protein. Changes in analyte concentrations or activities, followed the same trend in both groups, thus suggesting typical features of the newborn calf maturation. The first 24 hours after birth seem to represent a temporal key point in the newborn calf's life for switching from maternal dependence to a self-sufficient and independent survival. This study confirms that age-specific values should be considered for precise interpretation of laboratory results of newborn calves.

### HIGHLIGHTS

- Biochemical profiles of Holstein-Friesian and Belgian-Blue newborn calf in the first 48 hours of age are investigated.
- Almost all biochemical parameters change according to calf age in both groups and following the same trend in the two groups.
- Age-specific reference values should be considered during newborn calf evaluation.

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## Introduction

Immediately following birth, newborns of all species must adapt to their new environment. The result of these adaptation mechanisms is mirrored by marked changes in biochemical blood parameters and may depend on different factors (Hillman et al. 2012). Previous investigations on serum biochemical adaptations in calves (Mohri et al. 2007; Pérez-Santos et al. 2015) did not include the very first hours after birth, although it is exactly during these few hours that many metabolic adaptations take place (Panzani et al. 2012; Graves and Haley 2013). The reference intervals established for adult cattle are used to assess the biochemistry results of calves older than one week, but

at younger ages, events associated with birth and colostrum intake must be considered, as they might cause physiologic variations at least in some analytes (Pérez-Santos et al. 2015).

There are many studies reporting serum biochemical values in neonatal calves (Dubreuil and Lapierre 1997; Hugi and Blum 1997; Egli and Blum 1998; Knowles et al. 2000; Kühne et al. 2000), but in those studies different cattle breeds were enrolled, and therefore the effects of intrinsic calf factors like breed and attitude are still debated. Presently in Europe, two cattle breeds of great economic importance are the Holstein-Friesian (HF) and the Belgian Blue (BB), the first being explicitly reared for milk production,

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whereas the latter being intended for beef production and frequently used for crossbreeding because of its excellent carcass quality (Keane 2003; Fiems 2012). These two breeds are characterised by different growth impetus and muscularity, by differences in respiratory system dynamics (Lekeux et al. 1984; Gustin et al. 1987, 1988; Amory et al. 1994; Rollin et al. 1997), and by differences in some hematological values at birth (Probo et al. 2012).

Therefore, the aim of the present study was to investigate changes in biochemical parameters in newborn HF and BB calves during the first 48 hours of life, and to detect potential differences in trend changes between the two groups.

## Material and methods

### Animals

The study was conducted in Northern Italy ( $45^{\circ}28' = N$  and  $9^{\circ}41' = E$ ), and in the Western part of Belgium ( $51^{\circ}00' = N$  and  $03^{\circ}44' = E$ ), and approved by the Ethical Committees of the Universities of Ghent (EC 2010/12) and Milan (N 24/12).

A total number of 42 newborn calves were enrolled. Calvings in HF cows were supervised in order to detect any abnormality or difficulty. Parturitions occurring preterm (before 210 days of gestations) (Blowey and Weaver 2003) or requiring manual or pharmacologic assistance were not included. According to these criteria, 19 HF calves were enrolled. Twenty-three double-muscled BB calves born by elective caesarean section (Kolkman et al. 2010) were also included. Placental expulsion time was recorded, and placental retention was defined as no expulsion of the foetal membranes within 24 h of calving (Fourichon et al. 2000).

For all 42 newborn calves, the following data were recorded after birth: sex, weight, rectal temperature, maturity (Karapinar and Dabak 2008) and evidence of malformations. The interval between birth and sternal recumbency (TSR) (Schuijt and Taverne 1994) and the interval between birth and standing (TSU) (Mee 2004) were used to evaluate calf viability. For the same purpose, a modified Apgar score (Probo et al. 2012) was recorded within 10 min after birth. An Apgar index  $\geq 7$  was considered as normal. Clinical data registered at birth were used to assess the health status of the calves enrolled in the study.

Immediately after birth, each calf was transferred to an individual box with straw, and fed with 4 L of colostrum within 6 hours after birth and again twice/day on the second and third day after birth.

### Blood samples

A blood sample was taken from the jugular vein within 30 min after birth (and before first colostrum intake); plastic tubes with clot activator (Venosafe -VF-109SP, Terumo) and single-use needles with holders were used. Sampling was performed again 24 and 48 hours after birth (before daily colostrum administration). After collection, samples were immediately centrifuged at  $1000 \times g$  for 20 minutes and serum was stored at  $-20^{\circ}C$  until analysis.

A basic panel of biochemical tests for cattle was performed on serum samples using an automated spectrophotometer (ILAB300 plus, Instrumentation Laboratory S.p.a.) at the Veterinary Teaching Hospital of the University of Milan (Lodi, Italy). Specifically, the following parameters were evaluated: albumin (bromochresol green method), aspartate aminotransferase (AST; kinetic IFCC method), calcium (Ca; ortho-cresolphthalein method), phosphate (P; molybdate method), creatinine (Jaffè method),  $\gamma$ -glutamyl transferase ( $\gamma$ GT; kinetic IFCC method), glucose (GOD-POD method), urea (urease method), total protein (TP; biuret method). Chloride (Cl), sodium (Na) and potassium (K) were measured using ion-selective electrodes (ISE method, included in the ILAB300 plus instrument).  $\beta$ -hydroxybutyrate (BOHB) and glutamate dehydrogenase (GLDH) were measured by kinetic enzymatic methods (Randox Laboratories Ltd.). For all parameters except BOHB and GLDH, quality control was performed with two levels of human control serum samples (SeraChem Control Level 1 and 2, Instrumentation Laboratory S.p.a.) before each assay, and calibration was performed with human-based calibrators (ReferrIL G, Instrumentation Laboratory S.p.a.). For BOHB and GLDH, quality control was performed with 2 levels of bovine control serum samples (Bovine Assayed Sera Level 2 and 3, Randox Laboratories Ltd.). Inter- and intra-assay coefficients of variation for all the methods did not exceed 5%.

### Statistical analysis

Mean  $\pm$  SD of each variable was calculated, and data were analysed with Analyse-it software (Analyse-it<sup>®</sup> v2.21 Software Ltd). A non-parametric ANOVA for unpaired data (Kruskal-Wallis) was used to compare the different sampling times within each group of calves for each parameter. Differences were considered as significant if  $p < .05$ .

### Results and discussion

In the present study, biochemical parameters from healthy HF and BB calves were assessed in the first

48 hours from birth to verify age-associated changes, and to detect possible differences in trend changes.

All pregnancies were uncomplicated, all deliveries were at term, and there were no cases of placental retention. The HF calves comprised 9 males and 10 females, while there were 12 male and 11 female BB calves. All calves were born alive, viable and mature.

Most of the biochemical parameters showed significant alterations from 30 minutes after birth to 24 and 48 hours thereafter, while few parameters remained stable. In both groups, significant decreases were recorded at 24 and/or 48 hours compared with 30 min for albumin, calcium, chloride and creatinine, while significant increases were found for AST,  $\gamma$ GT, bilirubin,

GLDH, glucose and total protein. Mean  $\pm$  SD levels of biochemical parameters from birth to 48 hours after birth in both HF and BB calves are reported in Table 1.

In neonatal calves, there are marked alterations of biochemical traits which partly depend on the time of first colostrum intake as well as on the amount and quality of ingested colostrum (Egli and Blum 1998; Pérez-Santos et al. 2015). Moreover, the potential influence of the type of delivery must be considered in view of previous results (Probo et al. 2012).

The rise of the AST activity registered at 24 and 48 hours in both groups could partially be due to colostrum intake (Kurz and Willett 1991; Hammon and Blum 1998). The aspartate aminotransferase, in fact, is

**Table 1.** Mean  $\pm$  SD serum values of biochemical parameters in the 42 newborn calves.

	Time after birth	Holstein-Friesian	Belgian Blue
Albumin, g/L	30 min	28.000 $\pm$ 2.300 <sup>g</sup>	27.800 $\pm$ 1.700 <sup>g</sup>
	24 h	26.000 $\pm$ 2.300 <sup>e</sup>	24.100 $\pm$ 1.500 <sup>e</sup>
	48 h	27.000 $\pm$ 1.400 <sup>c</sup>	25.800 $\pm$ 1.500 <sup>i</sup>
Total protein, g/L	30 min	47.500 $\pm$ 3.700 <sup>d</sup>	46.200 $\pm$ 3.200 <sup>g</sup>
	24 h	57.300 $\pm$ 15.400 <sup>h</sup>	71.500 $\pm$ 8.020
	48 h	63.300 $\pm$ 13.200 <sup>i</sup>	71.500 $\pm$ 8.200 <sup>i</sup>
Urea, mmol/L	30 min	3.590 $\pm$ 1.090	3.370 $\pm$ 1.390
	24 h	3.660 $\pm$ 1.170	2.800 $\pm$ 0.890 <sup>b</sup>
	48 h	4.590 $\pm$ 2.500	3.430 $\pm$ 0.950
Creatinine, $\mu$ mol/L	30 min	243.980 $\pm$ 78.770 <sup>g</sup>	405.760 $\pm$ 132.600 <sup>g</sup>
	24 h	159.120 $\pm$ 66.300 <sup>e</sup>	211.610 $\pm$ 40.660 <sup>h</sup>
	48 h	154.700 $\pm$ 86.630 <sup>i</sup>	164.420 $\pm$ 19.450 <sup>i</sup>
AST, U/L	30 min	16.600 $\pm$ 6.470 <sup>g</sup>	13.370 $\pm$ 4.490 <sup>g</sup>
	24 h	57.140 $\pm$ 38.140 <sup>h</sup>	59.090 $\pm$ 12.920 <sup>h</sup>
	48 h	42.500 $\pm$ 26.090 <sup>i</sup>	46.200 $\pm$ 10.020 <sup>i</sup>
$\gamma$ GT, U/L	30 min	13.740 $\pm$ 4.650 <sup>g</sup>	16.840 $\pm$ 8.880 <sup>g</sup>
	24 h	1338 $\pm$ 1139	1410 $\pm$ 1052
	48 h	976 $\pm$ 672.300 <sup>i</sup>	1138 $\pm$ 623.500 <sup>i</sup>
GLDH, U/L	30 min	3.980 $\pm$ 3.300 <sup>g</sup> <sup>b</sup>	6.40 $\pm$ 3.810 <sup>g</sup>
	24 h	8.070 $\pm$ 4.660 <sup>h</sup>	12.070 $\pm$ 5.650
	48 h	5.160 $\pm$ 4.160	14.920 $\pm$ 8.380 <sup>i</sup>
Bilirubin, $\mu$ mol/L	30 min	3.420 $\pm$ 1.810 <sup>g</sup>	5.640 $\pm$ 2.560 <sup>g</sup>
	24 h	11.970 $\pm$ 7.010	10.430 $\pm$ 4.100
	48 h	11.620 $\pm$ 10.090 <sup>i</sup>	9.570 $\pm$ 4.440 <sup>i</sup>
Glucose, mmol/L	30 min	4.010 $\pm$ 2.200 <sup>a</sup>	3.290 $\pm$ 1.060 <sup>g</sup>
	24 h	5.230 $\pm$ 2.090	6.020 $\pm$ 1.110
	48 h	4.950 $\pm$ 1.320 <sup>c</sup>	5.540 $\pm$ 1.090 <sup>i</sup>
BOHB, mmol/L	30 min	0.006 $\pm$ 0.003	0.005 $\pm$ 0.002 <sup>d</sup>
	24 h	0.007 $\pm$ 0.004	0.006 $\pm$ 0.004
	48 h	0.007 $\pm$ 0.004	0.007 $\pm$ 0.002 <sup>i</sup>
Ca, mmol/L	30 min	2.920 $\pm$ 0.330 <sup>d</sup>	2.910 $\pm$ 0.280 <sup>g</sup>
	24 h	2.740 $\pm$ 0.300	2.780 $\pm$ 0.400
	48 h	2.770 $\pm$ 0.380	2.730 $\pm$ 0.190 <sup>i</sup>
P, mmol/L	30 min	1.960 $\pm$ 0.240	1.850 $\pm$ 0.350 <sup>g</sup>
	24 h	1.950 $\pm$ 0.320	2.110 $\pm$ 0.260
	48 h	2.000 $\pm$ 0.770	2.170 $\pm$ 0.250 <sup>i</sup>
Cl, mmol/L	30 min	97.570 $\pm$ 1.710 <sup>a</sup>	98.340 $\pm$ 2.720 <sup>g</sup>
	24 h	96.480 $\pm$ 2.780 <sup>e</sup>	95.680 $\pm$ 3.680
	48 h	94.900 $\pm$ 4.170 <sup>c</sup>	95.980 $\pm$ 3.060 <sup>f</sup>
Na, mmol/L	30 min	134.710 $\pm$ 2.780	137.170 $\pm$ 3.410
	24 h	133.130 $\pm$ 2.620	134.230 $\pm$ 4.910
	48 h	130.310 $\pm$ 5.040	135.300 $\pm$ 3.720
K, mmol/L	30 min	4.640 $\pm$ 0.410	4.200 $\pm$ 0.350 <sup>d</sup>
	24 h	4.700 $\pm$ 0.430	4.560 $\pm$ 0.360
	48 h	4.600 $\pm$ 0.800	4.640 $\pm$ 0.260 <sup>i</sup>

<sup>a</sup>Differences between 30 min and 24 hours after birth within each group.

<sup>b</sup>Differences between 24 hours and 48 hours after birth within each group.

<sup>c</sup>Differences between 30 min and 48 hours after birth within each group.

<sup>a,b,c</sup> $p < .05$ ; <sup>d,e,f</sup> $p < .01$ ; <sup>g,h,i</sup> $p < .0001$ .

a key enzyme in gluconeogenesis, but newborn calves are functionally monogastric and rely to a lower extent on this metabolic pathway to maintain glycaemia in comparison to weaned calves (Kaneko et al. 2008).

In both groups,  $\gamma$ GT activity significantly increased at 24 hours of age in comparison with birth, while no further increases were found from 24 to 48 hours after birth. Colostrum of cows is characterised by high  $\gamma$ GT activity (Vacher and Blum 1993) so that a higher  $\gamma$ GT activity in post-suckling calves was to be expected, and the measurement of serum  $\gamma$ GT activity has been proposed as an indicator of colostrum intake in newborn calves (Weaver et al. 2000). Also, the huge increase of GLDH serum levels registered at 24 hours after birth in both groups of calves was probably due to colostrum intake, as suggested by Hammon and Blum (1998).

The increase in bilirubin levels was evident at 24 and 48 hours when compared to birth sample in both groups. In the liver of newborns, there is a very low concentration of ligandin, so its ability to excrete bilirubin is limited (Klinkon and Ježek 2012). Moreover, birth is associated with destruction of foetal haemoglobin, finally resulting in higher serum bilirubin concentrations when compared with adults (Klinkon and Ježek 2012). Interestingly, bilirubin levels at birth appear to be lower in comparison to previous investigations (Mohri et al. 2007; Klinkon and Ježek 2012).

Increases in total protein concentrations from birth to 24 and 48 hours samples in both groups may reflect colostrum intake but also the maturation of the protein synthesis system. It has been reported that newborn calves, especially those that have not ingested colostrum, tend to have lower total protein concentrations than adults (Russell and Roussel 2007), as confirmed in both groups by the present data.

Serum albumin concentrations partially reflect hepatic synthesis (Mohri et al. 2007). The decreasing albumin levels detected in all calves from birth to 24 hours may reflect the immaturity of both the hepatic anabolic pathways and kidney glomerular function, typical for newborns. With time, hepatic maturation should lead to an increased albumin production, while kidney maturation should reduce albumin loss, thus increasing serum albumin levels, as seen in our data at 48 hours after birth in both groups.

The decreasing trend in serum creatinine levels registered in both groups seems to confirm this hypothesis. Some authors (Miall et al. 1999; Weintraub et al. 2015) hypothesised that the high serum creatinine concentration found in newborn babies immediately after birth merely reflects the maternal levels. Later on,

tubular reabsorption of creatinine seems to be responsible for its continually high plasma levels (Guignard and Drukker 1999). With time, maturational renal changes will impose a barrier to creatinine, so that from that point onwards, total body muscle mass, glomerular filtration rate, and tubular secretion will determine the serum creatinine values of the individual (Matos et al. 1998). Based on these studies, decreasing serum concentrations of creatinine in newborn calves could originate from the gradual maturation of kidney functions. The achievement of stable levels of creatinine within 48 hours from birth, indicates the completion of the adaptive processes of the kidneys to extra-uterine life. When considering potential maternal influences on serum creatinine levels of the newborn, the different maternal muscle mass between breeds should also be taken into consideration, though placental transfer of creatinine in ruminants is still a matter of debate (Pérez-Santos et al. 2015).

The increased urea levels detected in BB calves have no biological relevance since values are within normal ranges reported for calves (Mohri et al. 2007).

Lower glucose levels were registered at birth in both groups. Hypoglycemia at birth is a common and well-known situation, and newborns are highly dependent on glucose intake, and their carbohydrate stores are limited and quickly depleted, leading to mobilisation of fat (Keller et al. 1998). The glucose increases registered in both groups at 24 hours after birth, together with the absence of biologically significant changes in BOHB over time, show the importance of a prompt colostrum administration to avoid hypoglycaemia and fat mobilisation.

Changes in electrolyte levels through time, when present, occurred between birth and 24 hours of age. With this regard, we refer to the fact that the transition from foetal to newborn life is associated with major adaptations in water and electrolyte homeostatic control (Lorenz 1997), so that changes registered in calves at birth possibly reflect the transition from a maternal- to a foetal-controlled homeostasis.

In the present study, identical management systems were adopted for the two groups of calves, and blood sampling at birth was always done prior to the first colostrum intake, thus reducing possible confounding factors to colostrum quality, breed or to the type of delivery. Whatever the reason of possible differences in the absolute values of biochemical parameters, the changes detected in the present study followed the same trend in both groups of calves. Therefore, it is possible to conclude that these changes are proper of the newborn maturation, and that are analogue



between calves of different breeds or born by different types of delivery or fed with different colostrum.

## Conclusions


Significant changes in biochemical parameters were detected during the first 48 hours after birth in newborn calves; therefore, age-specific reference values should be considered for precise interpretation of biochemical results in the newborn calf. Moreover, the same trend of changes was detected between two different groups of calves (different breed and different type of delivery), thus possibly representing the consequence of the adaptive process of the newborn to the extra-uterine life.

Based on the present data, the first 24 hours after birth appear to represent a temporal key point in the newborn calf's life for switching from maternal dependence to a self-sufficient and independent survival.

## Disclosure statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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