Udruženje Bujatričara Srbije

Serbian Buiatric's Association

XIII Middle European
Buiatric's Congress

CONGRESS PROCEEDINGS

Hotel M (Best Western), Belgrade
Republic of Serbia
5-8 June 2013

Congress Proceedings
XIII Middle European Buiatric's Congress
Best Western Hotel M, Belgrade, Serbia, June 5 - 8, 2013

Publisher:	Serbian Buiatric's Association, Faculty of Veterinary Medicine, University of Belgrade
Chief Editors:	Prof. dr Dragan Gvozdić dr Branko Petrujkić
Prepres and technical editor:	BBN Congress Management d.o.o., 9 Deligradska, Belgrade
Cover Design:	Marija Marković
Printing Office:	Naučna KMD, Belgrade
Circulation:	230

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

636.2/.3(082)
614.9(082)
637.1(082)

MIDDLE European Buiatric's Congress (13 ; 2013 ; Beograd)
Congress Proceedings / XIII Middle European Buiatric's Congress, Belgrade, Serbia, June 5-8, 2013 ; organizers Serbian Buiatric's Association [and] Faculty of Veterinary Medicine [and] University of Belgrade ; chief editors Dragan Gvozdić, Branko Petrujkić. - Belgrade : Serbian Buiatric's Association : Faculty of Veterinary Medicine : University, 2013 (Belgrade : Naučna KMD). - 596 str. : ilustr. ; 24 cm

Tiraž 230. - Bibliografija uz svaki rad.

ISBN 978-86-916767-0-4 (SBA)
1. Serbian Buiatric's Association (Beograd)
a) Преживари - Зборници b) Ветерина - Зборници c) Млеко - Зборници
COBISS.SR-ID 198690572

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HEMATOLOGY AND BIOCHEMISTRY PARAMETARS IN NEONATAL CALVES WITH LOW IRON LEVELS

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ABSTRACT

Serum iron (Fe) deficiency is a common problem in newborn calves in dairy industry. Iron deficiency have an influence on the immune status of calves as well as on erythropoesis and thrombopoesis. Therefore, the aim of this study was to determine Fe status in neonatal calves and to relate Fe status to basic hematological and biochemical parameters and serum fibrinogen levels. Blood was collected from 21 calves of Holstein-Friesian breed (7 female and 14 male) 12 to 24 hours after birth. Calves were divided into two groups. Group A included calves with iron concentration less than 14 μmol/L and group B calves with concentration greater than 14 μmol/L. Iron concentration, total iron binding capacity (TIBC), fibrinogen, albumin and total protein were determined using standard laboratory techniques. Leukocyte count was determined using manual and PCV using microhematocrit method. The platelet count and differential leukocyte count was obtained by blood smears examination. The results have shown that all animals at birth had iron deficiency without signs of anemia. Platelet count was higher in group B. TIBC was significantly higher and transferrin saturation was significantly lower in group A. Fibrinogen concentration, other blood elements and globulin concentration were within the previously reported values for neonatal calves. Based on these results, we supposed that concentration of iron can influence platelet count in healthy neonatal cattle. Further studies are needed to confirm these findings and to establish its physiological significance.

KEY WORDS: iron deficiency, platelets, fibrinogen, neonatal calves

INTRODUCTION

Congenital iron (Fe) deficiency is a common problem in newborn calves in dairy cows industry in Serbia (Prodanović *et al.*, 2010; Katić, 2011). Fe deficiency and consequent neonatal anemia are considered as important predisposing factors for loss of adequate immunity and development of different pathology (Tennant *et al.*, 1975). Fe deficiency could be the cause of enhanced platelet count in humans (Schloesser *et al.*, 1965; Park *et al.*, 2012). Other reported cause of increased platelet count in horses and dogs is the presence of subclinical or clinical inflammation (Sellon *et al.*, 1997; Neel *et al.*, 2012). Further more, inflammation in cows is characterized with increased fibrinogen concentration (Hirvonen and Pyörälä, 1998; Latimer *at al.*, 2003).

There are no extensive literature data dialing with platelet count in newborn calves. Platelet count is an important factor that enables adequate haemostatic mechanisms in the body. Yet, it is not known if there is any relationship between iron concentration and platelet count in

neonatal calves. The aim of this study was to compare platelet count, fibrinogen concentration and other hematological and biochemical parameters in calves born with low and very low iron concentration.

MATERIALS AND METHODS

The experiment was conducted on 21 Holstein-Friesian calves (7 female and 14 male). All calves were vital at the time of birth and took colostrum before blood sampling. From each calf, jugular blood samples were taken in vacutainer tubes (EDTA D-VAC, DemophoriusUK, EU; BD Vacutainer, BD, Plymouth, UK and Na-citrate Vacuette, GreinerBio-One) 12 to 24 hours after birth. Hematological analyzes were performed 2 hours after blood sampling along with making a blood smear (Hemacolor®, Merck). Citrate and whole blood were centrifuged 15 minutes at 2500 rpm. Plasma and serum were separated and kept at -20°C until the time of analysis.

White blood cells (WBC) count was assessed using manual method. The platelet count and differential leukocyte count was obtained by blood smears examination (Harvey et al., 2001). Fibrinogen concentration was determined by Clauss method (Clauss, 1957). Standard commercial kits (Bioanalytica, Beograd, Srbija) were used for assessment of iron concentration, TIBC, total protein and albumin concentration (Spectrophotometer - RAYTO-1904C). Transferrin saturation (%) was calculated as follows = (Fe ×100%) / TIBC. Globulin concentration was calculated from total protein and albumin concentration. Packed cell volume (PCV) was obtained with microhematocrit method. After all analysis performed, using 14μmol/L as a treshold, we formed a group A (n=15) having an average of 7.2 ± 0.6 μmol/L (calves with very low Fe concentration) and a group B (n=4) having an average of 15.4 ± 0.6 μmol/L of iron (calves with low Fe concentration) in serum.

Data analysis was performed in Excel. Data are presented using descriptive statistic parameters. Differences between the variables were assessed using the Student's *t* test.

RESULTS AND DISCUSSION

Our results demonstrated that 12 to 24 hours after calving all neonatal animals had iron concentration below previously refered values (Bostedt *at al.*,1990). Only two calves had iron concentration of 16.6μmol/L and two calves 14 μmol/L (group B), while all the other animals had lower values (Table 1). Previously, it has been suggested that 16.1 +/- 1.9 μmol/L is a mark of latent iron deficiency in calves, predicting development of iron deficiency and influencing their vitality (Bostedt *at al.*,1990). Presented data indicate that all calves in our study were born with iron deficiency, yet without apparent anemia (Figure 1A). Anemia is not expected at birth, but when newborns are iron deficient, anemia develops during first few weeks of life, if parenteral iron supply is omitted (Bunger *et al.*, 1980). Iron deficit in calves from our study was related to low iron concentration of their mothers during prepartal period (Katić, 2011). When other hematological and biochemical data were compared between two groups of calves, the next results were obtained: PCV and WBC values as well as differential WBC count showed no difference (Figure 1A and 1B, Table 2), while platelet count was higher within group B (Figure 1C). All hematological findings were in accordance with published average values for this category of animals (Lumsden *et al.*, 1980). Higher platelet count in group B is in contrast with our expectations and previous results demonstrating that adult humans with iron deficiency have enhanced platelet count (Schloesser *et al.*, 1965; Park *et al.*, 2012). We can not exclued the possibility that platelet count in examined calves was also influenced by other variables that were not measured. It was recently evidenced that there are developmental differences in thrombopoiesis among neonatal and adult humans (Liu and Sola-Visner, 2011). As this is the first time that platelet count has been analised in iron deficient calves and that both parameters

could be related to calf health, we consider that our future research should be performed on wider number of animals and measurement of different variables in aim to obtain better insight in neonatal homeostatic mechanisms.

Table 1. Descriptive statistics data concerning iron values in two groups of calves.

groups	Fe concentration n = 15 >14 μmol/L	 n = 4 <14 μmol/L
mean±SE (min-max)	15.4±0.6 (14.1-16.6)	7.2±0.6 (2.7-10.5)

Table 2. Descriptive statistic data (mean±SE and min-max) concerning differential leukocyte formula in two groups of calves.

× 10^9/L	Groups according to different Fe concentration n = 15 <14 μmol/L	 n = 4 >14 μmol/L
Segmented NG	7.0 ± 1.1 (1.0-14.9)	4.0 ± 1.1 (2.2-7.0)
Band NG	0.8±0.6 (0.2-2)	0.5±0.5 (0.04-1.2)
Eosinophils	0.03±0.06 (0-0.2)	0.03±0.04 (0-0.09)
Lymphocytes	1.3 ±1.2 (0.2-4.8)	1.4±1.4 (0.2-4.7)
Monocytes	0.4±0.3 (0.05-0.9)	0.3±0.2 (0.04-0.4)

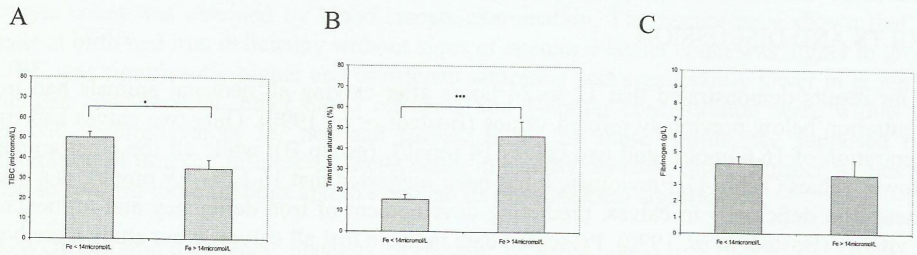

Figure 1. TIBC, tranferrin saturation and fibrinogen concentration between two groups of calves. Data are presented as mean±standard error.

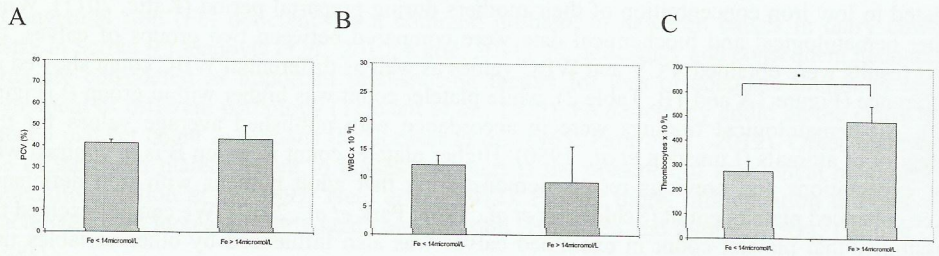

Figure 2. Hematological values between two groups of calves. Data are presented as mean±standard error.

Our data also demonstrated that TIBC and transferrin saturation were different among two groups (Figure 2A and 2B). These results are expected as low iron concentration stimulates transferrin synthesis (Skrzypczak *et al.*, 2009). As TIBC was higher and iron concentration lower in group A, transferrin saturation was also significanlty lower in group A. Indeed, transferrin saturation in group A was 16%, while in group B was 46%. Transferrin saturation of 46% is in upper reference limits for adult animals (Lumsden *et al.*, 1980). Calves in both groups had similar PCV, total protein and globulin concentration (Figure 2A and Figure 3A and 3C) indicating that the amount of consumed colostrum probably was similar between two groups. Concentration of albumin was lower in group of calves with higher iron concentration (Figure 3B). In both groups, all calves (except one) had fibrinogen concentration (Figure 2C) in the reference range for cattles (1 to 6 g/L - http://www.merckmanuals.com) indicating that calves did not have an acute phase response that could affect iron concentration.

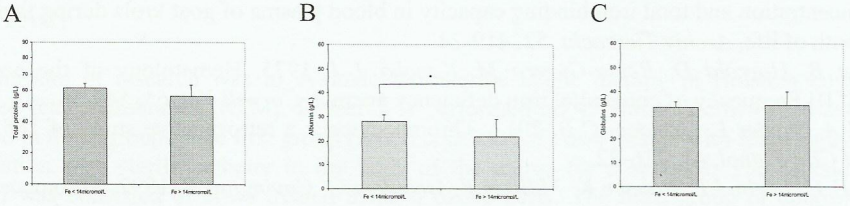

Figure 3. Protein values between two groups of calves. Data are presented as mean±standard error.

Concerning all data thogether, we can conclude that it is possible that iron concentration positivly affects platelet count in neonatal calves, but as multiple factors affect hematological and biochemical parameters soon after birth, larger study is needed to evaluate significance of this finding.

ACKNOWLEDGEMENTS

This work was supported by Ministry of Science, Republic of Serbia (grant No. 175061)

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