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*Original article*

# Biological variations of hematologic and biochemical parameters in cows during early lactation

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## Abstract

The aim of the study was to determine biologic variations of the main parameters in cows in early lactation and to compare intra-individual variations of parameters in cows and other species. 50 cows were included in the experiment. Blood samples were taken at the moment of calving, then on 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 28<sup>th</sup> day after calving. CVi had the following values: 1.9-5% for MCV, MCH, MCHC, GLU, TProt, ALB, UREA, Ca, P and Mg; 5.1-10% for RBC, Hgb, Hct, WBC, NEU, LYM, BHB, ALT, CHOL, TGC and >10% for PLT, NEFA, TBil, AST and GGT. For RBC, Hgb, Hct, MCV, MCH subject-based reference values or the reference change value should be used. For all other parameters except AST, population-based reference intervals should be used with caution. For LYM, NEU, PLT, GLU, TProt, ALB, CHOL and TGC index of individuality was close to 0.6 which means that subject-based reference values can be much more adequate to use than population-based reference intervals. For AST population-based intervals should be used. There is little effect of parity (increase CVi with parity) and no effect of milk production. CVi values obtained in cows in early lactation are specific because they deviate from the CVi values of other animal species and human. Calculated RCVs varied from 5.5% for MCHC to 41% for AST. High individuality index for many metabolic parameters opens possibility for development of model for longitudinal monitoring of metabolic status of individual animals during lactation. That requires further research.

**Key words:** cow, biological variability, biochemistry, hematology, individuality index

## Introduction

Peripartal period in cows is significant period for evaluation of metabolic, health and production status of cows. In this period negative energy balance occurs and lactation starts. That causes many metabolic changes in the organism (Cincović et al. 2018, Lakić et al. 2018, Benedet et al. 2019). Dynamical changes in dairy cows in peripartal period are determined by frequent blood sampling. Blood samples should be taken weekly or daily. Many parameters can be determined from these samples. Comparison of evaluated results during time showed that metabolism in cows express certain 'metabolic plasticity' in adaptations and that cows express different response to negative energy balance (Gross and Bruckmeier 2019). Classification of cows according to lipolysis and ketogenesis intensity, changes in body condition, ratio of catabolic and anabolic indicators etc. showed significant differences in hematologic and biochemical parameters in cows in first weeks of lactation (Hachenberg et al. 2007, Kessel et al. 2008, González et al. 2011, Cincović et al. 2012, Belić et al. 2018). Intra- individual differences in response to metabolic stress in early lactation open possibility that besides population-based reference intervals, subject-based reference values could be used in longitudinal monitoring of each animal during certain time in order to evaluate plasticity of metabolism.

Determinations of intra-individual variations are basis for determination of metabolic parameters reference values for each animal (animal-based reference value) (Walton 2012). If intra-individual variations are lower than population variations, evaluation process of metabolic status should consider intra-individual values. If intra-individual variations are similar to those in population, interpretation should consider inter-individual reference values of population. For this purpose, the index of individuality and subject-based referent values (reference change value, RCV) were calculated (Fraser 1989, 2004).

The aim of this study was to determine variations of important blood parameters in cows in early lactation and to compare intra-individual variation of blood parameters of cows with other species.

## Materials and Methods

Animals: 50 cows were included in the experiment. Blood samples were taken at the moment of calving, on 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 28<sup>th</sup> day after calving. Only healthy cows were included in experiment. Feeding was conducted according to NRC standards, two times per day. Water was given *ad libitum*. Blood sampling did not cause any stress in cows and didn't affect daily routine.

Blood sampling: Blood was taken from *v. coccigea*. Blood was collected at 06-08 h am. Blood samples were taken into tubes that contained EDTA - for hematologic analyzes and separation gel - for biochemical analyzes. Immediately after collection blood samples were delivered to Laboratory for Pathophysiology, Department of Veterinary Medicine Novi Sad.

Lab analyzes: Hematologic analyzes were conducted by hematology analyzer Nihon Kohden Celtac 6552. These analyzes were done two times from each sample and were presented as mean value of these two measurements. Red blood cell count (RBC), haemoglobin (Hgb), haematocrit (Hct), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red cell distribution width (RDW) and total leukocyte (WBC) count, neutrophyle count (NEU) and lymphocyte count (LYM) were determined. Glucose, NEFA, BHB, total protein, albumin, urea, Ca, P, Mg, total bilirubin, AST, ALT, GGT, cholesterol and TGC concentrations were determined by biochemical analyzer Chemray (Rayto, Ch). Standard biochemical kits (Randox, UK, Biosystem, SP) were used. Everyday internal quality control and monthly external quality control is standard procedure in our laboratory (delta check analysis, bias analysis, six sigma procedures with quality control plot evaluation).

Statistical analysis: CV between cows (between-subject variation,  $CV_G$ ), CV within cow (within-subject variation,  $CV_i$ ), analytical CV (analytical variation,  $CV_A$ ), index of individuality (IOI) and reference change value (RCV) were calculated. Standard formulas and data obtained in our laboratory were used (Freeman et al. 2017). Relations of  $CV_i$  of cows in early lactation with the same values in dogs, cats, horses and people were determined according to data obtained from scientific research and worlds bases. It was determined if  $CV_i$  will be changed in function of parity (lactation 1, 2-3 and >3 of cow) and in function of milk yield (<20, 20-30, >30L/day). Experiment included 16 cows in first, 17 cows in second and 17 cows in third lactation. Cows were divides into 3 groups by milk production in first 5 week of lactation (15 cows <20, 19 cows 20-30 L/day and 16 cows >30L/day).

## Results

Within-subject, between-subject, and analytical variation were established for each hematology and biochemistry parameter. These parameters were used to determine indices of individuality and RCV. Results are presented in Table 1 and 2.

$CV_i$  had the following values: 1.9-5% for MCV, MCH, MCHC, GLU, TProt, ALB, UREA, Ca, P and Mg; 5,1-10% for RBC, Hgb, Hct, WBC, NEU, LYM,

Table 1. Biological variation data for hematology parameters in cows during early lactation.

	Mean	Within cow CV <sub>i</sub>	Between cow CV <sub>G</sub>	Analytical CV <sub>A</sub>	IOI	RCV% (95% probability)
RBC×10 <sup>12</sup> /L	6.1	5.4	9.2	1.91	0.58	16.1
Hgb g/L	98	5.3	10.1	2.11	0.52	15.6
Hct n/n	0.31	6.1	10.4	1.62	0.56	17.7
MCV fL	50.2	2.3	7.6	1.13	0.35	7.1
MCH pg	17.1	1.9	6.6	2.21	0.34	8.1
MCHC g/L	343	3.3	3.2	1.32	0.92	5.5
WBC×10 <sup>9</sup> /L	8.3	9.1	10.5	3.21	0.87	26.7
NEU×10 <sup>9</sup> /L	4.8	9.4	13.1	5.42	0.72	30
LYM×10 <sup>9</sup> /L	3.5	7.9	12.6	6.09	0.63	27.7
PLT×10 <sup>9</sup> /L	458	11.2	15.7	8.19	0.75	38.5

Table 2. Biological variation data for serum biochemistry parameters in cows during early lactation.

	Mean	Within cow CV <sub>i</sub>	Between cow CV <sub>G</sub>	Analytical CV <sub>A</sub>	IOI	RCV% (95% probability)
NEFA mmol/L	0.53	12.6	10.2	2.11	1.24	35.4
BHB mmol/L	0.65	9.5	10.2	1.51	0.93	26.7
GLU mmol/L	2.8	4.4	7.2	1.9	0.61	13.3
TProt g/L	74.22	4.5	6.5	1.92	0.69	13.5
ALB g/L	33.82	3.9	5.9	2.1	0.66	12.3
UREA mmol/L	5.43	4.5	5.8	1.62	0.78	13.2
Ca mmol/L	2.15	3.5	3.1	1.71	1.13	10.8
P mmol/L	1.69	2.2	2.3	1.63	1.13	10.7
Mg mmol/L	1.14	2.4	2.5	1.82	1.04	8.5
TBil μmol/L	5.89	11.5	12.6	1.55	0.91	32.2
AST U/L	86.8	14.6	10.2	2.26	1.43	41.0
ALT U/L	24.8	9.5	12.6	2.31	0.75	27.1
GGT U/L	25.1	10.2	13.3	2.14	0.77	28.9
CHOL mmol/L	3.15	9.5	15.5	1.62	0.61	20.2
TGC mmol/L	0.22	8.3	12.1	1.93	0.69	23.6

BHB, ALT, CHOL, TGC and >10% for PLT, NEFA, TBil, AST and GGT.

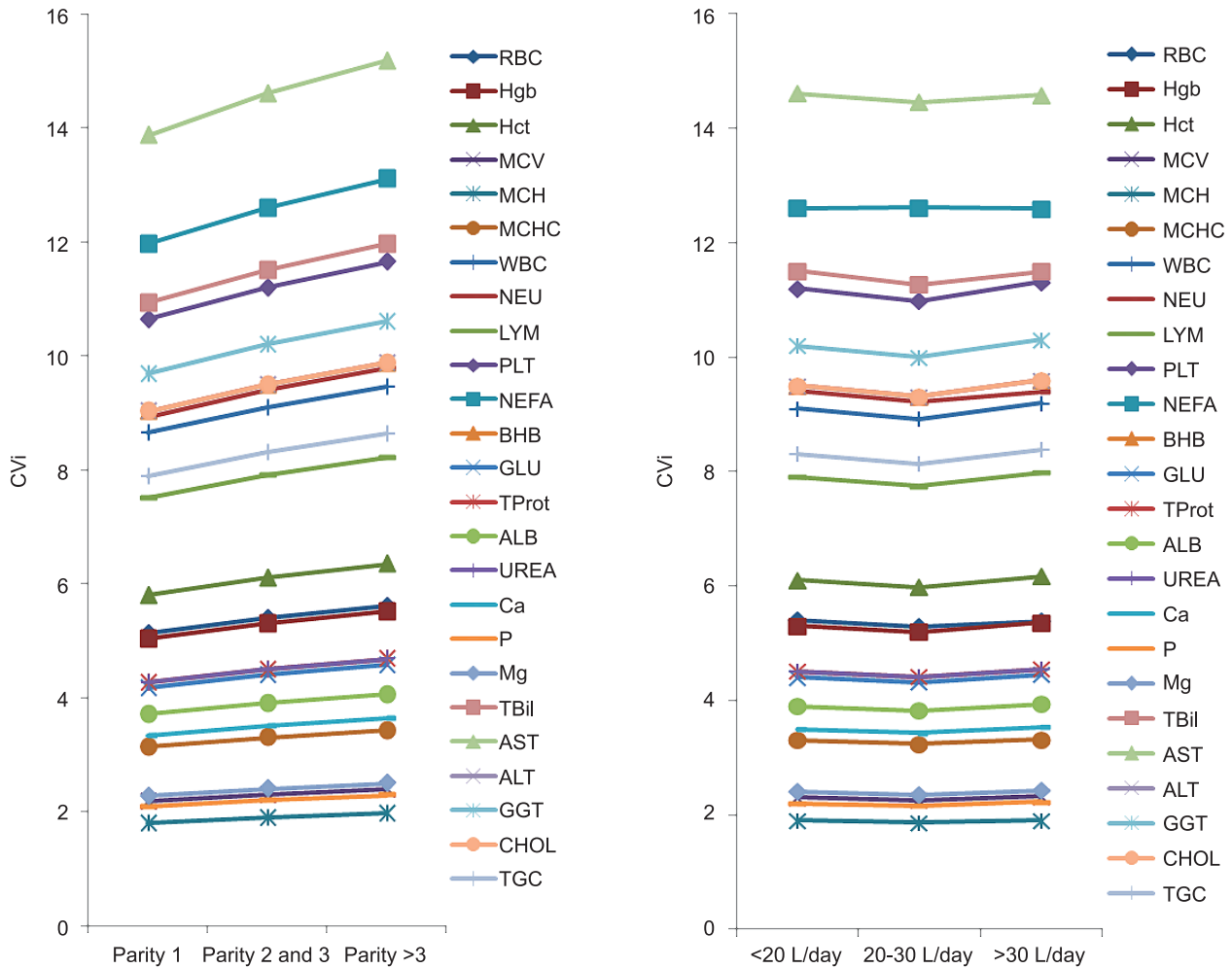
For RBC, Hgb, Hct, MCV and MCH index of individuality was low (<0.6), which means that subject-based reference values or the reference change value should be used. For all other parameters except AST, IOI was 0.6-1.4 which means that population-based reference intervals should be used with caution. For LYM, NEU, PLT, GLU, TProt, ALB, CHOL and TGC index of individuality was close to 0.6 which means that subject-based reference values could be much more adequate for use than population-based reference intervals. For AST high index of individuality IOI>1.4 was found which means that population-based intervals should be used.

The analysis performed on the basis of parity and

milk production is shown in Figs. 1 and 2, where it can be seen that there is little effect of parity (increase CV<sub>i</sub> with parity) and no effect of milk production.

The results show a good correlation between CV<sub>i</sub> values for different laboratory parameters in cows in our trial and in other species. However, by analyzing the line of identity on the charts, it can be concluded that the CV<sub>i</sub> values obtained in cows in early lactation are specific because they deviate from the CV<sub>i</sub> values of other animal species. The results are shown in Fig 3.

Calculated RCVs varied from 5.5% for MCHC to 41% for AST. RCVs were lower for red blood cell parameters, higher for leukocytes and the highest for PLT. For biochemistry parameters, RCVs were lower for macro-elements and glucose proteins, and higher for NEFA and liver markers.



Figs. 1 and 2. Influence of parity and milk production on CVi in cows during early lactation.

### Discussion

Different factors affect values of hematologic and biochemical parameters. These factors are: age, lactation period, health, milk yield, season of the year, breed etc. (Cozzi et al. 2011, Roland et al. 2014). Cows were included in experiment by random choice. Reduction of accidental variations and errors in interpretation of CVi can be provided by obtaining equal experimental conditions. In our experiment cows were kept in the same environmental conditions, feeding regimes and nursing. The common characteristic of all cows was period of lactation – early lactation, first month. Providing the same environmental condition and biological characteristics of animals is very important because it has been shown that CVi can vary depending on which type of animal is used experimental or randomly picked animals (Baral et al. 2014, Trumel et al. 2016). Among group uniformity, precision of laboratory which does analyzes has great significance. For maximum number of parameters high precision is reached, so CVA:CVi was  $\leq 0.5$ . Freeman et al. (2017) defined

that “when CVA:CVi is  $\leq 0.5$ , a minimum of 10-15 study subjects is needed from which specimens are obtained weekly over at least 4-6 weeks”. A larger number of cows was included in our research. This level hasn’t been reached in some high variable parameters like PLT, LYM and GR. Reason for this is existence of many interference that affect values of blood elements during measurements by hematologic analyzer (Operator manual for Nihon Kohden).

There are not many data about biological variability in cattle. Three experiments are well known and they were published in the last decade of the previous century (Jensen et al. 1991, 1992, 1992a). Jensen et al. (1991) examined critical difference between 2 analytical results for the red blood cell count (RBC), the white blood cell count (WBC), the hemoglobin concentration (Hb), and the hematocrit (PCV) in blood from Red Danish Dairy cows. Research of Jansen et al. (1992, 1992a) showed CVi for the main biochemical parameters in cows. CVi values in these experiments are lower compared to our CVi values. Reason for this can be that our research included cows in early lactation. In this

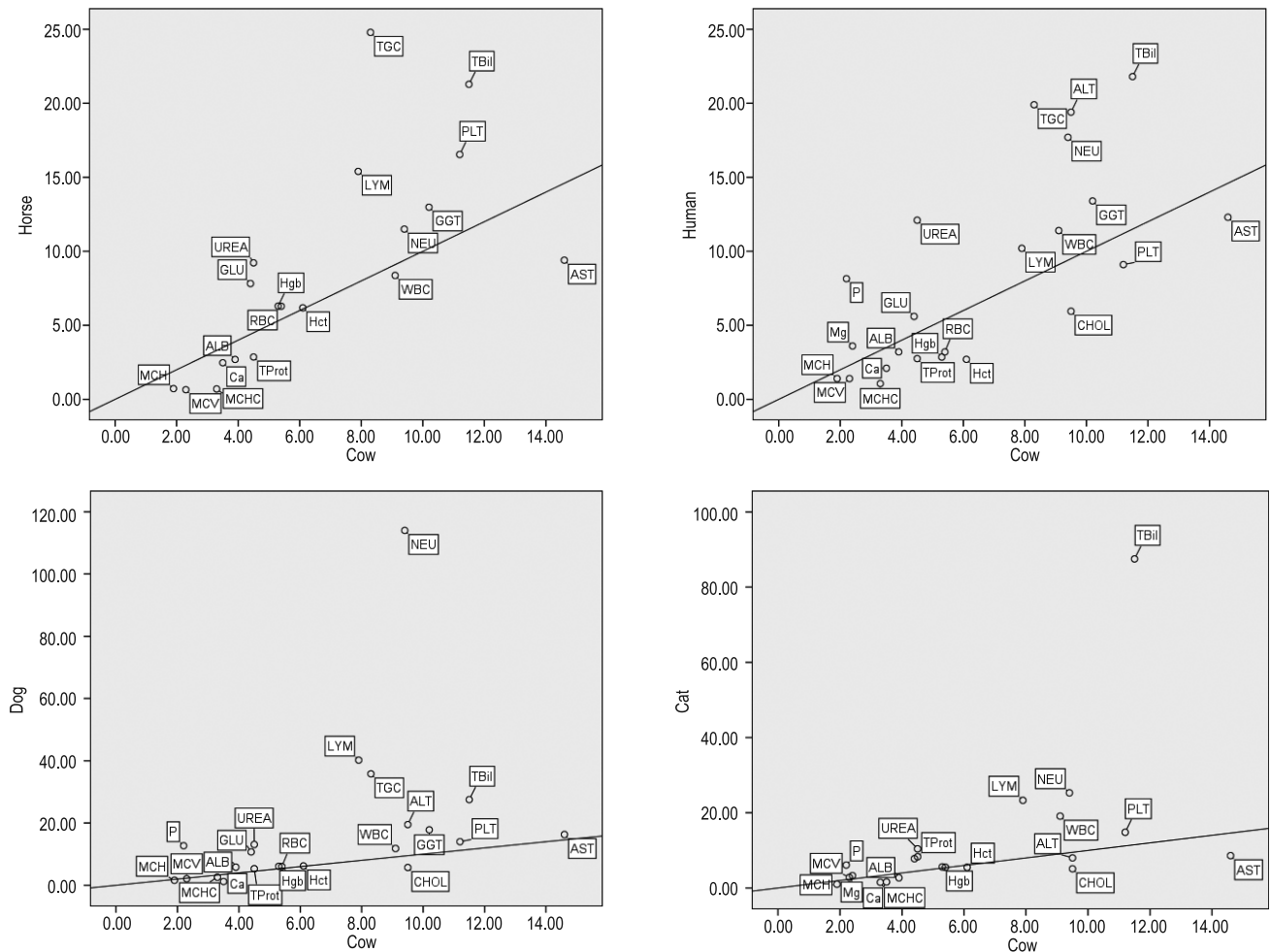


Fig. 3. Relationship between CVi in cows and horse, human, dog and cat for different blood parameters. Deviation between cow and other species for each individual parameter is represent in function of line of identity (full line).

period great metabolic disturbances occur. Listed researches haven't included cows in peripartal period. Time distance in research should not be neglected considering that one study of metabolic parameters during 1987-2004 showed that metabolic deviations of cows during time are greater. Reason for that is negative energy balance, energy deficit and disturbance of liver hepatocyte that affect cows during time (Kayano and Kida 2015). Experiments of Jansen et al. (1992, 1992a) were performed during 5 days or 5 weeks. In these two studies great difference of CVi has been noted, so it can be concluded that similar research hasn't gotten similar data variability. Besides, in these experiments CVa is often 0 or near 0. This can be a consequence of using data from double analyzed materials, but not long termed deviations from referent materials that we have used in our research. CVi weren't affected by milk yield in our experiment, but slightly higher CVi for many parameters in higher compared to lower parity was noted. Multiparital cows showed greater metabolic imbalance in peripartal period (Piñeyrúa et al. 2018). That can cause increase of CVi with increasing parity.

In our research CVi of 2-16% was noted, while in dogs, cats, horses and humans for the same parameters CVi are 2-25%. The greatest number of metabolic parameters showed greater CVi in examined species compared to cows in our experiment. However, minor variations were noted in parameters like AST, cholesterol, Hgb, HCT, MCHC, Ca and TPProt in examined species compared to cows. These data indicate existence of specific intra- individual variations of parameters in cows compared to other examined species. The highest individuality was noted for red blood cells parameters, Ca and Tprot in our research and this is similar for other four species. Great variability followed by reduced individuality was noted for liver enzymes, leucocytes (GR and LYM), bilirubin and PLT in our research and this is similar for four other species (Ruaux et al. 2012, Baral et al. 2014, Bourgès-Abella et al. 2015, Falkenö et al. 2016, Wright et al. 2019, Jones 2019).

Some parameters showed large intraindividual variation probably due to metabolic rearrangement and stress response in early lactating cows. Metabolic rearrange-



ment is characterized by lipolysis and increased hepatocyte load (manifested through values of NEFA, BHB, AST, ALT, GGT, CHOL, TGC), and stress leads to a change in the leukogram of cows (WBC, NEU, LY), so high CVi for the listed parameters may be a consequence of magnitude change in the value of these parameters in the weeks after calving. Also, the dispersion of reference intervals for these parameters is wider than other examined parameters, so the range and magnitude of intraindividual variation in healthy individuals is greater. For the same reason, the intraindividual variation for macro-elements (Ca, P and Mg) is the lowest, because the dispersion of their reference values is smaller, and homeostatic mechanisms maintain their concentration in a narrow range of values.

Calculation and understanding of intra-individual variability in cows during early lactation is of great significance. These data allow us to see following aspects (Ricós et al. 2009): how to set quality specifications for analytical performance and evaluate the clinical significance of changes in consecutive results from an individual, how to assess the usefulness of population-based reference values and determine which sample is optimal for analyzing a specific constituent. Also, how to select the best test among several for a specific clinical purpose (eg, diagnosis, monitoring) and the most informative units of expression for each analyte for reporting results, how to determine the number of analyses needed to establish an individual's homeostatic set point, and the last, how to validate new procedures in a laboratory. Delta check values, that are highly specific and sensitive indicators of sample origin, were determined in our laboratory. In that way it can be determined if two samples are from the same animal (Cincović, unpublished). Further research should examine usage possibility of biological variability of cows in everyday practice during evaluation of health and productivity status at farms.

In conclusion, careful interpretation of metabolic and hematologic parameters is necessary in cows in early lactation. For RBC, Hgb, Hct, MCV, MCH index of individuality was low (<0.6), which means that subject-based reference values or the reference change value should be used. For all other parameters except AST, IOI was 0.6-1.4 which means that standard population-based reference intervals should be used with caution. For AST we found high index of individuality IOI>1.4 which means that population-based intervals should be used. High individuality index for numerous metabolic parameters opens up possibility for longitudinal monitoring model development for each cow during lactation. That requires further research.

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