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A COMPARISON OF THE CONCENTRATIONS OF ENERGY-BALANCE-RELATED VARIABLES IN JUGULAR AND MAMMARY VEIN BLOOD OF DAIRY COWS WITH DIFFERENT MILK YIELD

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The aim of this study was to compare the concentrations of blood variables obtained simultaneously from the jugular and mammary veins of dairy cows. Eighty Holstein cows were divided into four equal groups: dry, low- (LY), medium- (MY) and high-yielding (HY). Blood insulin, glucose, non-esterified fatty acid (NEFA), beta-hydroxybutyrate (BHBA) and urea concentrations were measured. The jugular and mammary vein (J/M) ratio between concentrations of each variable was calculated. Differences between the groups of cows in concentrations of variables in the jugular vein were not in accordance with those obtained for the mammary vein. J/M values for insulin and glucose concentrations were above 1.0 in all groups of cows. The ratios for NEFA and BHBA concentrations were under or equal to 1.0 in dry and LY cows but above 1.0 in the MY and HY groups, indicating that in MY and HY cows those metabolites are apparently utilised by the mammary gland. J/M values for urea were above 1.0 in dry and LY cows but less than 1.0 in groups MY and HY, indicating that in the latter case urea is apparently released by the mammary gland. In conclusion, J/M for NEFA, BHBA and urea may be useful for estimation of the critical point when the mammary gland receives insufficient energy precursors for its current activity.

Key words: Energy balance, mammary vein, jugular vein, dairy cow, milk yield

Energy balance (EB) is calculated as the difference between energy consumption in the diet and energy use by the body to support maintenance, growth, milk

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production and reproduction. High-yielding dairy cows experience negative energy balance (NEB) in early lactation, because feed intake cannot provide the required energy for milk yield and maintenance. However, there are large differences between animals in the magnitude of NEB, which is important, due to the association of EB with milk production, health and fertility (Le Blanc, 2010; Gumen et al., 2011).

Blood variables like glucose, non-esterified fatty acid (NEFA), beta-hydroxybutyrate (BHBA) and urea concentrations are widely used indicators of EB status (Cozzi et al., 2011). With the onset of lactation and the associated NEB, glucose concentration in the blood, which is essential for milk lactose synthesis, decreases. Simultaneously, NEFA and BHBA concentrations increase, to provide additional energy for maintenance and milk production. Among the wide variety of interrelated parameters, circulating urea concentration is influenced by the amount and rumen degradability of dietary carbohydrate and can be a good indicator of energy supply to the cow (Laven et al., 2007). Namely, the ammonia produced through protein degradation in the rumen can be used by the rumen microflora only if the amount of readily fermentable carbohydrates is sufficient. The physiological balance is obtained with a ration containing 14% protein (Kirchgessner and Kreuzer, 1985), which is sufficient in most of the cases for a daily milk yield of 20 kg. With increasing milk production, the protein/energy ratio of feed gets higher, resulting in an increase of ammonia that cannot be used by the rumen bacteria. As urea production in the liver increases, an elevation of blood and milk urea concentrations follows (Oltner and Wiktorsson, 1983).

It is widely accepted that blood for the determination of variables to be used for the estimation of energy balance of early-lactation dairy cows, is sampled from the jugular vein. There are only few studies in which parameters for this purpose have been determined in blood taken from mammary or tail veins (González et al., 2011). It was noted that, in some cases, metabolite concentrations differ in blood from different veins, such as jugular and mammary, which may be attributed to the apparent mammary uptake or release of these substances (Gagliostro et al., 1991). Since the apparent mammary uptake of some blood metabolites is of crucial importance for estimating a cow's energy status, we postulated that determination of blood variables obtained simultaneously from mammary and jugular veins might provide additional information about the energy status of early-lactation cows. We calculated jugular/mammary venous ratios of several metabolites instead of measuring mammary arteriovenous difference (Cant et al., 1993; Rius et al., 2010), which is a widely accepted technique for the determination of mammary uptake of metabolites, since the latter cannot be performed in the practice when the metabolic status of cows is evaluated under farm conditions.

The aim of our study was to determine the apparent mammary uptake and release of some energy-balance-related blood variables in dry cows and cows in early lactation with different milk yields, to compare the concentrations of some blood analytes simultaneously in the jugular and mammary veins.

Materials and methods

Cow selection

Eighty clinically healthy Holstein cows were chosen from the dairy herd of a large commercial farm (PKB Corporation, Belgrade). The selected cows ranged from 4 to 6 years of age and were housed in a tie-stall barn. They were divided into four groups of equal size. The first group consisted of 20 dry cows at day 30 before the expected calving. The second, third and fourth groups each included 20 cows at day 30 of lactation, but with different milk yields: low-yielding cows (LY, 31 to 40 litres per day), medium-yielding cows (MY, 41 to 50 litres) and high-yielding cows (HY, more than 50 litres per day).

The animal-related component of the study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, University of Belgrade in accordance with the National Regulations on Animal Welfare.

Feeding management

The ingredients and the chemical composition of the diets for dry and early-lactation cows are listed in Tables 1 and 2. Regardless of milk yield, all cows received the same amount of feed for the first 30 days of lactation, as this is the feeding strategy applied at the commercial dairy farm where the experiment was carried out.

Table 1

Ingredients of the diets for dairy cows

Ingredient (kg/day)	During the dry period	Until day 30 of lactation
Alfalfa hay	3.00	3.50
Wheat straw	2.60	–
Alfalfa haylage	3.50	1.00
Corn silage	10.00	17.50
Extruded fullfat soybeans	–	2.00
Sugarbeet molasses	–	0.30
Dry sugarbeet pulp	–	0.75
Corn grain meal	1.45	3.79
Barley grain	0.27	0.90
Sunflower meal	0.85	3.51
Wheat flour	0.50	0.90
Dicalcium phosphate	0.04	0.10
Calcium carbonate	0.04	0.24
Sodium chloride (iodised)	0.02	0.13
Vitamin-mineral premix*	0.03	0.10

*Composition of vitamin-mineral premix: vitamin A (1,500,000 IU), vitamin D_3 (300,000 IU), vitamin E (4,000 mg), niacin (2,000 mg), biotin (20 mg), iron (3,000 mg), copper (1,200 mg), manganese (6,000 mg), zinc (5,000 mg), iodine (100 mg), selenium (30 mg), cobalt (40 mg), magnesium (5 000 mg), antioxidant (10,000 mg). The vitamin-mineral premix is manufactured by PKB Corporation, Belgrade, Serbia

Table 2

Ingredients of the diets for dairy cows

Chemical composition	During the dry period	Until day 30 of lactation
DM, kg/day	11.8	22.1
Net energy of lactation (NEL), MJ/day	72.0	148.2
Metabolisable protein (MP), g/day	940.0	2142.0
Rumen degradable protein (RDP), g/day	1110.0	2743.0
Rumen undegradable protein (RUP), g/day	388.0	1025.0
Crude fat, % DM	2.6	4.7
Non-structural carbohydrates	35.0	39.3
Acid detergent fibre (ADF), % DM	29.9	22.0
Neutral detergent fibre (NDF), % DM	44.7	33.2
NDF from forage, % DM	39.2	20.6
Ca, g/day	41.0	66.0
P, g/day	32.0	25.0
K, g/day	161.0	88.0

Milk yield and composition

Milk yields were measured at the morning and evening milking (6:00 and 18:00) using Milk Master equipment (De Laval, Australia). Milk samples were analysed for composition using Milko-Scan 33 (Foss Electric, Hillerod, Denmark). Average daily milk yields on day 30 of lactation were 32.75 ± 0.53 L (LY), 43.15 ± 0.59 L (MY) and 54.55 ± 0.86 L (HY). Differences in milk yields between the groups were significant ($P < 0.001$, respectively) and were expected due to the experimental design. Average milk fat contents were 3.71 ± 0.05% (LY), 3.63 ± 0.08% (MY) and 3.56 ± 0.07% (HY), and did not differ between the groups. Average milk protein contents were 3.25 ± 0.02% (LY), 3.19 ± 0.03% (MY) and 3.12 ± 0.05% (HY) and differed significantly between the LY and HY groups ($P < 0.01$).

Blood collection

Blood samples from each cow were taken simultaneously from the jugular and mammary veins 2 to 3 h after the morning feeding. Samples for insulin, NEFA, BHBA and urea analysis were obtained with a sterile needle into plain tubes and allowed to clot spontaneously for approximately 15 min. After centrifugation at 1,000 *g* for 20 min the serum was decanted and stored at −20 °C until analysed. Sodium-fluoride-oxalate tubes were used for glucose determination. Insulin was determined using a commercial RIA kit (INEP Zemun) intended for human sera but validated for bovine sera. Cross-reactivity of the anti-human insulin antibodies with bovine insulin was close to 100%. Standards for the radioimmunoassay were made from bovine insulin. Intra- and interassay co-

efficients of variation (CV) for the insulin concentrations were less or close to 10% and less than 5%, respectively. Concentrations of NEFA were measured using an enzymatic colorimetric method (Randox, Great Britain), BHBA by a NAD-dependent enzymatic UV method (Randox, Great Britain), urea by a fully enzymatic method (Human, Germany) and glucose by an enzymatic colorimetric method (Human, Germany). The samples were processed in a semi-automatic biochemical analyser (Stat Fax® 3300 Chemistry Analyzer, Awareness Technology, Inc., Palm City, FL, USA).

Statistical analysis

The results obtained were analysed statistically using Statistica v. 6 (StatSoft, Inc., Tulsa, OK, USA). As average values, arithmetic means were determined for homogeneous data and medians for heterogeneous values in a group. Since many data were not homogeneous (CV higher than 30%) or normally distributed, the significance of differences between average concentrations of blood variables in the jugular and mammary veins were evaluated using the Wilcoxon matched pairs test. The differences of average values of the analysed blood variables between groups of cows was computed using the Kruskal-Wallis test and the Mann-Whitney U-test.

Results

Jugular vein insulin concentrations were significantly lower in lactating cows than in dry cows (Table 3). In the LY, MY and HY groups, jugular insulin concentrations decreased by 27.15%, 42.05% and 49.32%, respectively, when compared to dry cows. Mammary vein insulin concentrations were significantly lower in lactating than in dry cows, and decreased by 28.72%, 44.11% and 65.13%, respectively, when compared to dry cows. The J/M ratio for insulin concentrations was above 1.0 in all groups of cows, and was significantly higher in Group HY than in all other groups. The Wilcoxon test showed significant differences between jugular and mammary insulin concentrations in each group of cows (Table 3).

Jugular vein glucose concentrations did not differ significantly between the groups of lactating cows, but were considerably lower in lactating than in dry cows (Table 4). Mammary vein glucose concentrations were significantly lower in all lactating groups than in the group of dry cows, and also decreased significantly with increasing milk yield. In Group HY, jugular glucose concentration was 83.58% of the value determined in dry cows, while mammary glucose concentration was 42.19% of the value determined in dry cows. The J/M ratios for glucose concentrations were above 1.0 in all groups of cows. The Wilcoxon test showed significant differences between jugular and mammary vein glucose concentrations in all examined groups of cows (Table 4).

Table 3

Insulin concentration in jugular and mammary veins and their ratio in dry and milking cows

Insulin concentrations (μU/L)		Group			
		DRY	LY	MY	HY
Jugular vein*	M_e	22.1[a]	16.1[b]	12.8[c]	11.2[c]
	LQ	14.9	14.5	10.6	9.5
	UQ	24.3	18.6	16.1	13.5
Mammary vein*	M_e	19.5[a]	13.9[b]	10.9[c]	6.8[d]
	LQ	13.2	11.2	10.0	5.6
	UQ	20.2	16.5	13.8	9.9
Wilcoxon test (P) jugular vs. mammary result#		P = 0.001	P < 0.001	P < 0.002	P < 0.001
Jugular/Mammary ratio*	M_e	1.15[a]	1.13[a]	1.18[a]	1.66[b]
	LQ	1.12	1.07	1.13	1.34
	UQ	1.20	1.19	1.23	1.91

M_e – median; LQ – lower quartile; UQ – upper quartile; *Values in a row of median values based on the Mann-Whitney U-test with different superscripts are significantly different ($P < 0.05$); #Significance levels of the Wilcoxon test between jugular and mammary concentrations

Table 4

Glucose concentration in jugular and mammary veins and their ratio in dry and milking cows

Glucose concentrations (mmol/L)		Group			
		DRY	LY	MY	HY
Jugular vein*	M_e	3.35[a]	3.05[b]	3.00[b]	2.80[b]
	LQ	3.15	2.65	2.50	2.45
	UQ	3.50	3.20	3.30	2.90
Mammary vein*	M_e	3.20[a]	2.85[b]	2.20[c]	1.35[d]
	LQ	3.00	2.55	1.80	1.10
	UQ	3.30	3.05	2.30	1.90
Wilcoxon test (P) jugular vs. mammary result#		P = 0.001	P < 0.001	P < 0.001	P < 0.001
Jugular/Mammary ratio*	M_e	1.05[a]	1.04[a]	1.47[b]	2.05[c]
	LQ	1.01	1.04	1.25	1.50
	UQ	1.06	1.07	1.68	2.41

See footnote to Table 3 for explanations

Jugular NEFA concentrations were significantly higher in lactating than in dry cows and also increased significantly with milk production between the groups of lactating cows (Table 5). Mammary NEFA concentrations were also

higher in lactating than in dry cows. The J/M ratios for NEFA concentrations were lower than 1.0 in Groups DRY and LY and above 1.0 in Groups MY and LY. Values of the J/M ratio increased significantly through Groups LY, MY and HY. The Wilcoxon test showed significant differences between jugular and mammary NEFA concentrations in all examined groups of cows, but the probability was borderline for Group MY (P = 0.0504; Table 5).

Table 5

Non-esterified fatty acid (NEFA) concentration in jugular and mammary veins and their ratio in dry and milking cows

NEFA concentrations (mmol/L)		Group			
		DRY	LY	MY	HY
Jugular vein*	M_e	0.16[a]	0.30[b]	0.50[c]	0.80[d]
	LQ	0.16	0.20	0.40	0.65
	UQ	0.31	0.40	0.75	0.80
Mammary vein*	M_e	0.28[a]	0.40[b]	0.50[c]	0.40[b]
	LQ	0.25	0.40	0.40	0.25
	UQ	0.33	0.40	0.75	0.40
Wilcoxon test (P) jugular vs. mammary result#		P < 0.001	P < 0.001	P = 0.0504	P < 0.001
Jugular/Mammary ratio*	M_e	0.69[a]	0.75[a]	1.07[b]	2.12[c]
	LQ	0.57	0.50	1.00	1.77
	UQ	0.90	1.00	1.27	2.67

See footnote to Table 3 for explanations

Jugular vein BHBA concentrations increased in the order DRY<LY< MY<HY (Table 6). Mammary vein BHBA levels were significantly lower in Group LY than in dry cows. The other groups of lactating cows (MY and HY) had similar BHBA concentrations in the mammary vein as dry cows. J/M ratios for BHBA concentrations were lower than 1.0 in dry cows, but equal to or higher than 1.0 in all lactating cows. The Wilcoxon test detected significant differences between jugular and mammary BHBA concentrations in all examined groups of cows except Group LY (Table 6).

Average jugular vein urea concentrations were not significantly different between the lactating groups of cows but were significantly higher in Groups LY and MY than for the dry group (Table 7). Mammary vein urea concentrations were considerably higher in lactating cows than in the group of dry cows and were 2, 2.63, and 3 times higher in the LY, MY and HY group, respectively, compared to values determined in dry cows . The J/M ratios for urea concentrations were higher than 1.0 in dry cows and in Group LY but lower than 1.0 in Groups MY and HY. Differences between the dry cow group and all lactating groups in urea J/M ratios

were significant with much higher ratios in dry and LY cows. As for the lactating groups, in MY and HY cows the urea J/M ratio was only 35% of the J/M value found in dry cows. The Wilcoxon test showed significant differences between jugular and mammary urea concentrations in all examined groups of cows except Group LY (Table 7).

Table 6

Beta-hydroxybutyrate (BHBA) concentration in jugular and mammary veins and their ratio in dry and milking cows

BHBA concentrations (mmol/L)		Group			
		DRY	LY	MY	HY
Jugular vein*	M_e	0.10[a]	0.20[a]	0.50[b]	0.80[c]
	LQ	0.10	0.10	0.30	0.60
	UQ	0.30	0.35	0.70	0.90
Mammary vein*	M_e	0.30[a]	0.20[b]	0.25[ab]	0.40[a]
	LQ	0.30	0.10	0.10	0.10
	UQ	0.35	0.25	0.45	0.60
Wilcoxon test (P) jugular vs. mammary result#		P = 0.001	P = 0.328	P < 0.001	P < 0.001
Jugular/Mammary ratio*	M_e	0.33[a]	1.00[b]	2.00[c]	1.90[c]
	LQ	0.33	0.87	1.62	1.44
	UQ	0.87	2.00	2.67	5.50

See footnote to Table 3 for explanations

Table 7

Urea concentration in jugular and mammary veins and their ratio in dry and milking cows

Urea concentrations (mmol/L)		Group			
		DRY	LY	MY	HY
Jugular vein*	M_e	2.0[a]	3.0[b]	3.0[b]	3.0[ab]
	LQ	2.0	3.0	2.0	3.0
	UQ	3.0	3.5	3.0	4.0
Mammary vein*	M_e	1.5[a]	3.0[b]	3.95[c]	4.5[c]
	LQ	1.0	2.0	3.9	4.0
	UQ	2.0	3.0	5.0	6.0
Wilcoxon test (P) jugular vs. mammary result#		P = 0.002	P = 0.118	P = 0.002	P < 0.001
Jugular/Mammary ratio*	M_e	1.75[a]	1.67[b]	0.67[c]	0.67[c]
	LQ	1.00	0.87	0.51	0.54
	UQ	2.00	1.50	1.00	0.83

See footnote to Table 3 for explanations

Discussion

The analysis of mammary gland metabolism by comparing concentrations of blood variables obtained simultaneously from the mammary and jugular veins has lost significance since the introduction of assessment using the simultaneous measurement of blood variables from the *a. intercostalis* and *v. subcutaneous abdominis,* i.e. determining the mammary arteriovenous difference (Hanigan et al., 1992; Cant et al., 1993; Rius et al., 2010). Although the composition of jugular blood does not exactly reflect arterial blood composition, close accordance with data based on mammary arteriovenous differences suggests that J–M differences can be used to evaluate general trends (Gagliostro et al., 1991). The main reason for using this approach to assess mammary gland activity in lactating cows is the fact that blood sampling from the *a. intercostalis* needs surgical preparation, which is not easy to perform in routine practice in the field. On the contrary, two veins are much more accessible for blood sampling.

The energy status of cows is usually determined by analysing concentrations of variables obtained only from jugular vein blood (Dyck et al., 2011). In our study, differences in concentrations of the examined variables in jugular vein blood were expected and are in accordance with earlier data (Kida, 2002; Šamanc et al., 2011). Namely, jugular vein insulin concentrations in lactating cows decreased with higher milk production due to the more pronounced NEB during early lactation (Hayirli et al., 2011). Jugular glucose concentrations also differed significantly between the groups of lactating cows but were within the physiological range in all cases (2.50–4.16 mmol/L, according to Kaneko et al., 2008). Glucose concentration is under tight homeostatic control and therefore is not a good indicator of the cow's energy status (Herdt, 2000). The concentration of jugular NEFA reflects the magnitude of lipid mobilisation from storage and is highly positively correlated with the extent of NEB in early-lactation cows. BHBA indicates the completeness of fatty acid catabolism in the liver. When the supply of NEFA to the liver exceeds its ability to catabolise fatty acids completely, ketone body production increases. Ketone bodies can be used by the muscles as an alternative fuel source to glucose, sparing glucose for milk production. Although jugular vein urea concentrations were significantly higher in Groups LY and MY compared to the group of dry cows, the levels were within the physiological range (3.33–4.99, according to Kaneko et al., 2008). Elevated urea concentrations usually indicate an excess of dietary protein relative to energy supply (Fekete et al., 1996; Tamminga, 2006).

The results obtained for concentrations of variables in the mammary vein indicated that there were differences from the trend of changes in the jugular vein. The jugular to mammary vein ratio (J/M) shows differences according to milk production more clearly. Namely, if the ratio is higher than 1.0, it indicates that the blood variable is apparently utilised in the mammary gland, while if it is

lower than 1.0 it indicates apparent mammary release of the metabolite. This interpretation of the results is a slightly modified version of that obtained by Gagliostro et al. (1991), who presented jugular to mammary vein blood differences considering that a positive result indicates that the blood variable is apparently utilised in the mammary gland. In all groups of cows in this study, J/M for insulin and glucose concentrations were higher than 1.0, meaning that those substances were apparently utilised by the mammary gland in all cases. J/M ratios for insulin were similar in dry, low- and medium-yielding cows but were significantly higher in high-yielding cows, indicating that the apparent mammary uptake of insulin was greater in those cows. This was expected due to the well-known physiological finding that, although without a direct impact on milk production, insulin is utilised in the lactating mammary gland for milk protein synthesis within bovine mammary epithelial cells (Menzies et al., 2009) and in high-yielding cows for mammary cell proliferation (Sorensen et al., 2006). The J/M for glucose was lower in dry and LY cows, while it was significantly higher in Groups MY and HY. This confirms our earlier work where simultaneous glucose concentrations in the *v. auricularis magna* and *v. subcutanea abdominis* were compared (Stamatović et al., 1983). Moreover, glucose is utilised by the mammary gland mainly for lactose synthesis and to supply ATP (through glycolysis and the TCA cycle), as well as for milk fat formation (Guinard-Flament et al., 2006).

The J/M ratio for NEFA concentrations in dry and LY cows was lower than 1.0, indicating that in those cows the mammary gland was sufficiently supplied with energy precursors and had no need to use endogenous sources for metabolic activity. J/M for NEFA concentrations in Groups MY and HY was higher than 1.0, suggesting that this metabolite is apparently utilised in mammary glands with higher milk production. The same explanation may be used to interpret the results for BHBA, which indicate an imbalance between mammary gland supply and its needs in cows producing more than 41 litres of milk per day.

The J/M ratio for urea concentrations was higher than 1.0 in dry and LY cows but lower than 1.0 in Groups MY and HY. This indicates apparent utilisation of this metabolite by the mammary gland in the dry period, while in cows with higher milk production there is an apparent release of urea. Although there is no exact explanation for this, it may be speculated that in MY and HY cows, when energy requirements of the mammary gland are enhanced, some amino acids may not be entirely used for protein synthesis, leading to increased urea concentrations and apparent release from the mammary gland, probably mainly due to the known action of arginase catalysing the conversion of arginine to ornithine and urea. We presumed the similarity of amino acid composition of jugular vein and arterial blood based on data presented by Hanigan et al. (1991) who reported similarities between coccygeal vein blood and arterial blood in amino acid composition.

In conclusion, measuring concentrations of variables in blood samples taken simultaneously from the jugular and mammary veins of cows should en-

able a more accurate assessment of the energy status of cows in early lactation than if such variables are determined exclusively in samples obtained from the jugular vein. This refers especially to concentrations of those parameters where the J/M ratio changes with increasing milk production from values lower than 1.0 to values higher than 1.0 (NEFA and BHBA) or, conversely, from values higher than 1.0 to values lower than 1.0 (urea). These changes for all three variables occur when milk production is higher than 41 litres. Determination of concentrations of those three metabolites simultaneously in jugular and mammary veins and calculation of their J/M ratios may have diagnostic value. Namely, a J/M ratio for BHBA and/or NEFA lower than 1.0 and a J/M ratio for urea higher than 1.0 indicate that the energy supply to the mammary gland is probably sufficient. On the contrary, when the J/M ratio for BHBA and/or NEFA is higher than 1.0 and the J/M ratio for urea is lower than 1.0, the energy supply to the mammary gland is insufficient for optimal mammary gland activity. In addition, since the J/M ratio for BHBA tended to change from less than 1.0 to more than 1.0 between Groups LY and MY, it may be speculated that this variable may be the one that is initially changed when the energy needs for milk production exceed the energy supply.

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