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CHANGES IN BLOOD VALUES OF GLUCOSE, INSULIN AND INORGANIC PHOSPHORUS IN HEALTHY AND KETOTIC COWS DURING AN INTRAVENOS GLUCOSE TOLERANCE TEST

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Abstract: The aim of the present study was to determine the degree of blood glucose utilization by peripheral tissue on the basis of changes in blood concentrations of glucose, insulin and inorganic phosphorus in healthy (n=8) and cows with ketosis (n=7) after intravenous infusion of glucose solution. After intravenous infusion of a total of 500 ml of 50 % of glucose solution, glucose and insulin blood values in both groups of cows increased significantly within 10 and 30 minutes of the experiment ($P < 0.05$). After intravenous infusion of glucose, it was established that values of inorganic phosphorus were decreased ($P < 0.05$) in blood in both groups of cows. Within testing period there was a significant decrease ($P < 0.01$) in the blood value of inorganic phosphorus in ketotic cows in comparison with healthy ones. This is linked with the active entry of glucose into the glucolytic pathway of peripheral tissues. It can thus be concluded that there is a higher degree of blood glucose utilization by peripheral tissues in ketotic cows.

Key words: ketosis, glucose utilization, glucolytic pathway, peripheral tissue

Introduction

The optimal supply of liver and mammary gland with glucose has an important role in preserving the health of dairy cows in the early stage of lactation.The first metabolic change in primary ketosis in dairy cows in early

lactation is hypoglycaemia. It causes serious metabolic changes in cow body, which are manifested through lipomobilization from body reserves and ketogenesis and lipogenesis in the liver (*Veenhuizen et al., 1991, Vazquez-Anon et al.,1994*). The glucose tolerance test is used for estimating the ability of the endocrine pancreas for synthesis and secretion of insulin in ruminants. The insulin concentration in blood is reduced in ketotic cows, compared to healthy animals in the early lactation, before and after infusion of a glucose solution (*Hove 1978, De Cupere et al., 1991, Sakai et al. 1996, Šamanc et al., 1996, Djoković et al., 2009*). Similar results have been reported by other authors (*Peters and Elliot 1984, Šamanc et. al., 1996, Djoković et al., 2007*), after an intravenous infusion of propionate solution, because propionic acid directly stimulates pancreatic secretion of insulin in ruminants.

During the glucose tolerance test, it is very difficult to estimate on the basis of glycaemia whether the metabolic disorder was induced by liver disease or hypofunction of the endocrine pancreas. At the same time, an estimation of the concentration of inorganic phosphorus in blood can be helpful. Namely, the decrease of its concentration in blood after intravenous infusion of glucose is linked with the active utilization of glucose into the glucolytic pathway of peripheral tissue, while a considerably smaller amount of glucose is used for glycogenesis in the liver (*Sakai et al., 1993, Gründberg et al., 2006, Djoković et al., 2007, Djoković et al., 2009)*.

The aim of the present investigation was to determine of blood glucose utilization by peripheral tissue on the basis of changes in blood concentrations of glucose, insulin and inorganic phosphorus in healthy and ketotic cows during an intravenous glucose tolerance test.

Materials and Methods

A total of eight healthy and seven cows with ketosis in the earliest stage of lactation (7-14 days post-partum) were chosen from a Holstein dairy herd. The diagnosis of ketosis was based on clinical symptoms (decreased appetite, rumen atony, behaviour changes), including high concentrations of β-hydroxybutyrate (2.60 ± 0.45 mmol/L) in the blood (>2.6 mmol/L; *Oetzel, 2004*) and ketone bodies in the urine. The Lestradet test was used to examine the presence of ketone bodies in the urine. Healthy cows did not show clinical symptoms of ketosis and urinary ketone bodies were not determined in those cows. The blood concentrations of BHB in healthy cows were 0.53 ± 0.18 mmol/L (> 1.2 mmol/L is frame for subclinical ketosis; *Oetzel, 2004*). Cows were of similar body mass (560-580 kg), 4-6 years old, an average of 3 lactations with a mean milk yield of 7750 L (calculated over 305 days) in the previous lactation. The meal was prepared in the way to meet the energy needs of animals in early lactation. Early lactation cows were fed a diet consisting of 7 kg lucerne hay, 20 kg maize silage (30% Dry Matter, DM), 5 kg concentrate (18% crude proteins, CP). Chemistry characteristic

of meal were: 87,15 MJ NEL; crude protein 13,58% of DM; rumen undegradable protein 35,91% of crude protein; fat 3,09 %DM, fibre 23,26% DM.

The test was carried out in the morning at 09 h about 3 h after feeding. A solution of glucose (500 mL of 50 %) was injected intravenously during 5 minutes into a jugular vein of each animal. Blood samples were taken from the opposite jugular vein before (0) and10, 30, 60, and 90 minutes after injection. Blood samples were allowed to clot for 3 hours at 4°C and then, they were centrifuged (1500g, 10 minutes), following which the serum was carefully harvested and stored at -20°C until analysis. Blood samples collected into fluoride-containing tubes were immediately centrifuged in the same manner, and plasma glucose levels were determined. The concentrations of insulin in the blood serum were determined by ELISA methods (Cusabio) using Rayto reader. Glucose (glucose oxidase test, GOT) and inorganic phosphorus (modificate classical phosphomolybdate method) were measured using a Randox kit and Rayto spectrophotometer.

Statistical analysis: Influence of time during intravenous tolerance test to concentration of glucose, insulin and inorganic phosphorus was analysed by ANOVA-procedure with posthock LSD test. For all purposes we used statistic software Statgraphic Centurion (Statpoint Technologies Inc.Warrenton, Va, Virginia, USA).

Results and Discussion

Basically, the glucose tolerance test is used to estimate the functional ability of the beta cells for the synthesis and release of insulin (*Hove 1978, Sakai et al.,1993, Sakai et al.,1996, Gründberg et al. 2006, Djoković et al.,2009*). Changes in glucose, insulin and inorganic phosphorus values in the peripheral circulation of the cows given glucose intravenously are shown in Figs 1, 2 and 3.

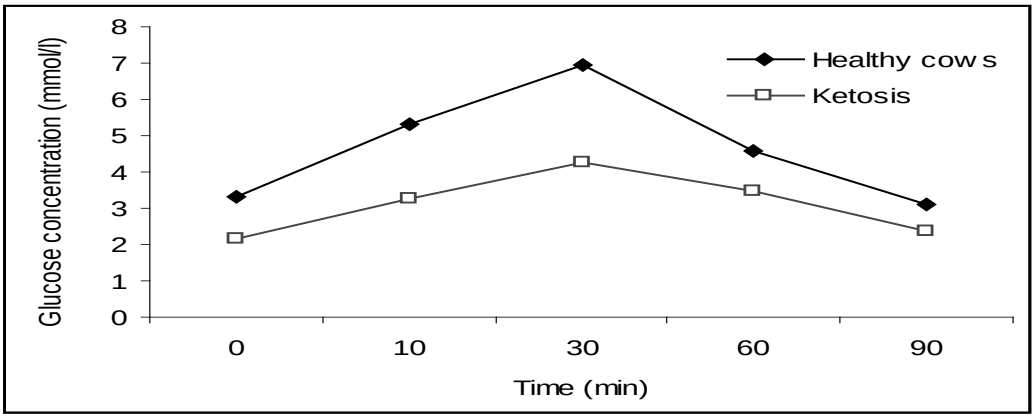

Figure 1. Changes in blood values of glucose of healthy and cows with ketosis after intravenous infusion of glucose solution

The initial blood glucose values in the healthy cows were within physiological range 2.5 - 4.2 mmol/L *(Radostits et al., 2000*), whereas in ketotic cows were determined hypoglycemia. The initial blood glucose values were significantly lower in ketotic cows (2.17 ± 0.16 mmol/L) than in healthy cows (3.30 ± 0.24 mmol/L; $P < 0.01$). Glucose injection led to a significant increase in glycaemia within 10, 30 and 60 min of the experiment in both groups of cows ($P < 0.05$), which peaked within 30 min and then slowly declined. In the group of cows with ketosis, the mean blood glucose value was significantly lower than in healthy group of cows during whole test period ($P < 0.01$). The fact that glucose values after intravenous administration showed similar increments and similar rates of decline in healthy and ketotic cows, indicated that the mean of glucose disposal was similar in both groups of animals. Thus, the pancreatic islets were subjected to almost identical glucose stimuli. These results are in accordance with previous data (*Hove 1978, Šamanc et al., 1996, Djoković et al., 2009*).

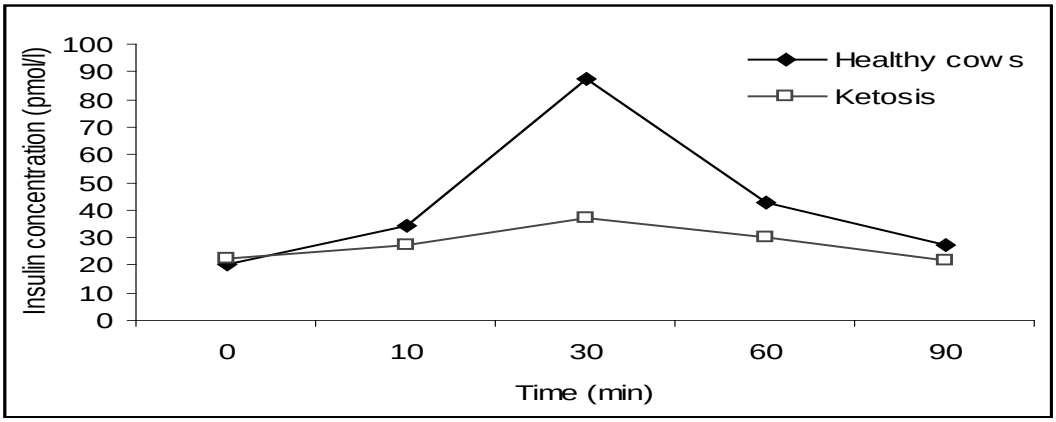

Figure 2. Changes in blood values of insulin of healthy and cows with ketosis after intravenous infusion of glucose solution

Mean initial blood insulin values were lower but without significant diferences ($P > 0.05$) in the healthy group (22.42 ± 2.67 pmol/L) than in the ketotic group of cows (20.37 ± 4.65 pmol/L). The glucose injection led to a significant increase ($P < 0.05$) in insulinemia within 10 and 30 minutes of the experiment in both groups of cows and confirm the possibility of glucose to influence the synthesis and release of insulin from beta cells of the endocrine pancreas. Namely, the value increased to 87.12 ± 24.47 pmol/L within 30 minutes in healthy cows compared with 37.00 ± 12.24 pmol/L in ketotic cows ($P < 0.01$). After 30 minutes blood insulin concentrations decreased in both group of cows and marked difference in insulinemia among groups was maintained within 60 and 90 minutes after glucose administration ($P < 0.05$). The low secretory responses of

insulin in ketotic cows, are therefore probably a result of a pancreas with a low secretory capacity for insulin, developed during the days or weeks of hypoglycemia which regularly accompanies high ketosis. The obtained results show the preserved function (relative insufficiency) of the beta cells of the endocrine pancreas of ketotic cows. Other authors have reported similar results (*Peters and Elliot 1984, De Cupere et al., 1991, Sakai et al., 1993, Sakai et al.,1996, Djoković et al., 2009*).

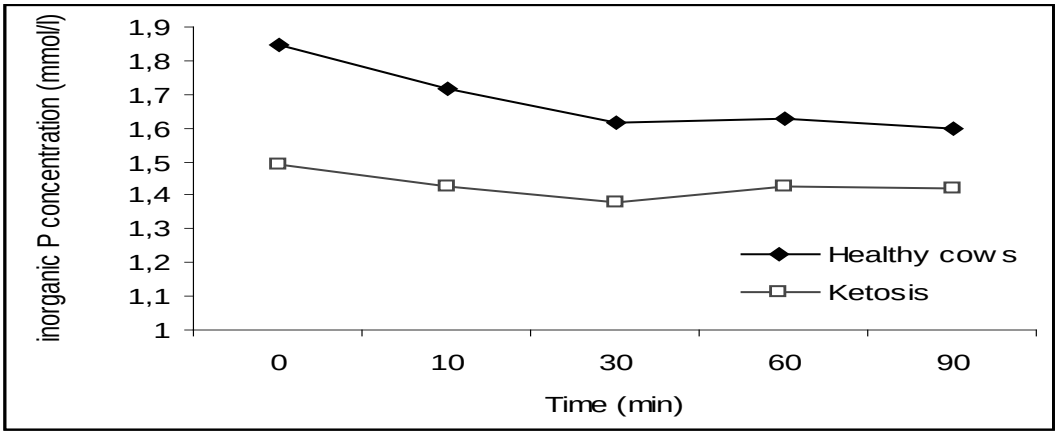

Figure 3. Changes in blood values of inorganic phosphorus of healthy and cows with ketosis after intravenous infusion of glucose solution

The inorganic phosphorus blood values in the healthy group of cows before the infusion of a glucose solution were 1.85 ± 0.12 mmol/L, whereas they were 1.48 ± 0.10 mmol/L in ketotic cows ($P < 0.01$). After intravenous infusion of a glucose solution, a decrease of the inorganic phosphorus values in the blood in both groups of cows were determined during the test period ($P < 0.05$). Among the tested groups of cows, statistically significant differences of the values of inorganic phosphorus in the blood were determined during the test period ($P < 0.01$). The significant decrease of the value of inorganic phosphorus in the blood of both groups of cows after glucose injection and a significant lower level in ketotic cows compared to the healthy ones, during whole testing period could be a sign of the increased usage of glucose in blood by the peripheral tissue in ketotic cows. Therefore, the view that blood glucose is used for energy purposes by peripheral tissue is confirmed. These results are in accordance with the previus observation (*Sakai et al. 1993, Gründberg et al. 2006, Djokovic et al. 2007,2009*).

Conclusion

- Significantly lower ($P < 0.01$) level blood inorganic phoshorus in ketotic cows compared to the healthy ones, during whole testing period could be a sign of the increased usage of glucose in blood by the peripheral tissue in ketotic cows.
- A solution of glucose (500 mL of 50 %) injected intravenously is an efficient therapeutic mean in treatment of ketosis in dairy cows

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Promene vrednosti glukoze, insulina i neorganskog fosfora u krvi kod zdravih i ketoznih krava za vreme intravenoznog opterećenja glukozom

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Rezime

Cilj ovog rada je bio da se proceni stepen iskorištavanja glukoze od strane perifernih tkiva na osnovu promena vrednosti glukoze, insulina i neorganskog fosfora kod zdravih (n=8) i ketoznih krava (n=7) nakon infuzije rastvora glukoze. Nakon intravenozne infuzije 500 ml 50% rastvora glukoze utvrđeno je da se vrednosti glukoze i insulina značajno povećavaju ($P < 0.05$) tokom 30 i 60 minuta eksperimenta. Nakon infuzije rastvora glukoze, vrednosti neorganskog fosfora su se značajno smanjivale ($P < 0.05$) u obe grupe krava tokom ispitivanog perioda.U okviru ispitivanog perioda utvrđene su značajno niže vrednosti ($P < 0.01$) inorganskog fosfora u krvi kod ketoznih krava u odnosu na zdrave krave. Smanjivanje vrednosti inorganskog fosfora u krvi dovodi se u vezu sa aktivnim ulaskom glukoze u periferna tkiva, pa se može zaključiti da je veći stepen iskorišćavanja glukoze od strane perifernih tkiva kod krava obolelih od ketoze u odnosu na zdrave krave tokom testa opterećivanja glukozom.

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