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Evaluating the metabolism of high producing Holstein dairy cows following bolus intravenous glucose administration

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ABSTRACT

Providing energy demands with emphasis on glucose supply can prevent metabolic disorders in high producing dairy cows. Hence, we hypothesized that bolus intravenous glucose administration may change the concentrations of circulating metabolic biomarkers in order to prevent and control metabolic dysfunctions in dairy cows. Twenty-five multiparous, high producing Holstein dairy cows were divided into 5 equal groups containing early, mid and late lactation, and far-off and close-up dry cows. All cows received dextrose 50 % intravenously at 500 mg/kg, 10 mL/kg/hour. Blood samples were collected from all animals prior to and 1, 2, 3 and 4 hours after dextrose 50 % infusion, and sera were separated to determine glucose, insulin, non-esterified fatty acid, β-hydroxybutyric acid, cholesterol, triglyceride, high, low and very low density lipoproteins. Significantly decreasing patterns of non-esterified fatty acid and β-hydroxybutyric acid were seen in early and mid-lactation and close-up dry cows after glucose administration ($P<0.05$). Rapid and significantly increasing patterns of glucose and insulin concentrations were seen in early and mid-lactation and close-up dry cows after glucose administration ($P<0.05$). In early lactation and close-up dry cows, insulin remained at high concentrations at hour 4. Despite the increased levels of insulin in early and mid-lactation and close-up dry cows, glucose concentrations did not decrease near to base line levels at the 4th hour. There were no significant changes to the patterns of the lipid profile after glucose administration ($P>0.05$). The results of this study demonstrated that bolus intravenous glucose infusion can influence metabolism in high producing Holstein dairy cows. The changing patterns of circulating metabolic profile indicated that glucose is an important direct controller of metabolic interactions and responses in dairy cows in various physiological states.

Key words: glucose, energy demands, metabolism promotion, Holstein dairy cows

Introduction

The glucose metabolism is of major interest in high producing dairy cows, because certain vitally important cell types and metabolic pathways have to rely on glucose

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as the only energy substrate (ASCHENBACH et al., 2010). The glucose metabolism in ruminants is different from other mammals. In ruminants, volatile fatty acids from the gastrointestinal tract are the major energy source, rather than direct sources of glucose. Amongst ruminants, high producing dairy cows occupy a special position regarding glucose metabolism which is related to increases in energy requirements driven by both fetal needs and lactogenesis (DE KOSTER and OPSOMER, 2013). A dairy cow has several and different physiological states during its life. A marked disproportion between dry matter intake and milk yield level, related to negative energy balance, takes place during some states (GRUMMER et al., 2004; ALLEN et al., 2005). Furthermore, in some states the gravid uterus consumes a high level of energy to maintain and develop the fetus (FIORE et al., 2014). The unique transition from pregnancy non-lactating to non-pregnancy lactating status can drive the metabolism to extremes, and the glucose metabolism has a key role in preventing metabolic dysfunction and diseases (DE KOSTER and OPSOMER, 2013; DJOKOVIĆ et al., 2013). The increased glucose requirements of the gravid uterus during late pregnancy and the even greater requirements of the lactating mammary glands necessitate major adjustments in glucose production and utilization in the maternal liver, adipose tissue, skeletal muscle, and other tissues (FIORE et al., 2014). The negative energy balance during some physiological states can increase lipolysis and decrease lipogenesis, causing high levels of non-esterified fatty acids (NEFA) and β-hydroxybutyric acid (BHBA) concentration, which are indicative of lipid mobilization and fatty acid oxidation (WATHES et al., 2009). Excessive fat mobilization can induce an imbalance in hepatic carbohydrate and fat metabolism, which may result in metabolic problems, such as ketosis and fatty liver syndrome (GOFF and HORST, 1997). Metabolic disorders can cause economic losses to the dairyfarmer due to treatment costs, decreased milk production, impaired reproduction efficiency and increased involuntary culling (NIELSEN and INGVARTSEN, 2004).

A positive balance between input and output of energy can prevent metabolic disorders in high producing dairy cows, and the intravenous administration of glucose has been recommended to prevent and treat metabolic abnormalities (RADOSTITS et al., 2007). Hence, we hypothesized that bolus intravenous glucose administration may change the concentrations of circulating metabolic biomarkers by providing a source of energy and the alteration of the cow's metabolism. Therefore, the aim of the present experimental study was to evaluate the changing pattern of glucose, insulin, NEFA, BHBA and lipid profiles as circulating metabolic biomarkers following bolus intravenous glucose administration in high producing Holstein dairy cows in various physiological states, and to compare them. The results of the present experimental study may suggest a preventive and therapeutic regimen of metabolic disorders in dairy cows.

Materials and methods

Animals. The present experiment was performed after being approved by the Ethics Committee of the School of Veterinary Medicine, Shiraz University. This research was carried out in the winter of 2014 on 25 multiparous Holstein dairy cows from a high producing industrial dairy farm around Shiraz, Southwest Iran. These cows were housed in open-shed barns, with free access to water and shade. The total mixed rations were formulated and prepared for all animals according to National Research Council (NRC) requirements. At this farm, a dry period of 60 days was considered. Milk production was about 10,000 kg per year, with an average of 3.6 % milk fat, and 3.3 % milk protein. All the animals were clinically healthy, had no history of debilitating disease, and were free from internal and external parasites due to routine anti-parasitic programs at this farm. The body condition scores (BCS) of these animals were estimated based on a 0 to 5 system. The cows were divided into 5 equal groups containing early (30.2 ± 5.7 days after calving, with 3.25 ± 0.25 BCS), mid (108.1 ± 8.4 days after calving, with 3.25 ± 0.25 BCS) and late lactations (184.5 ± 5.7 days after calving, with 3.5 ± 0.25 BCS), far-off (281.9 ± 5.4 days after calving, 228.4 ± 8.6 days of pregnancy, with 3.5 ± 0.25 BCS) and close-up dry periods (312.1 ± 8.3 days after calving, 255.6 ± 6.3 days of pregnancy, with 3.5 ± 0.25 BCS).

Bolus intravenous glucose administration. All experiments were performed one hour after the morning meal, at 6 a.m. approximately. A 14-gauge 5.1-cm catheter was secured in the right jugular vein and used for blood samplings and dextrose infusions. A blood sample was taken immediately after catheterization, and dextrose 50 % (Zoopha®, Parnian Co., Iran) was administered at 500 mg/kg, 10 mL/kg/hour subsequently (DE KOSTER and OPSOMER, 2013). Blood samples were collected from all cows through the fixed catheter prior to and 1, 2, 3 and 4 hours after dextrose 50 % infusion, in plain tubes.

Serological assays. Immediately after blood collections, the sera were separated by centrifugation for 10 minutes at 3,000 g and stored at -22 ºC until assayed. Glucose was assayed by the enzymatic (glucose oxidase) colorimetric method (ZistChem®, Tehran, Iran). Insulin was measured using a bovine insulin ELISA kit (Cusabio®, China, specificity 100 %, and precision: intra-assay and inter-assay CV<8 % and 10 %, respectively). BHBA and NEFA were assayed by the colorimetric method (Ranbut®, Ireland). The sera were analyzed for total cholesterol by a modified Abell-Kendall/Levey-Brodie (A-K) method (ABBEL et al., 1952; BURTIS and ASHWOOD, 1994), triglyceride (TG) by the enzymatic procedure of McGOWAN et al. (1983). Lipoproteins were isolated using a combination of precipitation and ultracentrifugation (at D.1006 for 16 h at 100,000 g in a Spinco 40.3 rotor). HDL-cholesterol was measured using the precipitation method. In the first step, the precipitation reagent (sodium phosphotungstate with magnesium chloride) was added to the serum to aggregate non-HDL lipoproteins which were sedimented by centrifugation (10,000 g for 5 min). The residual cholesterol was then measured by the enzymatic method (BURTIS and ASHWOOD, 1994). LDL-cholesterol was calculated as the difference between the total cholesterol measured in the precipitate and in the HDL fraction minus 0.2×triglyceride (LDL cholesterol = total cholesterol - HDL cholesterol - 0.2 × TG). VLDL-cholesterol was estimatedas one-fifth of the concentration of triglycerides (FRIEDEWALD et al., 1972).

Statistical analyses. All data are presented as mean ± standard deviation (SD). Differences among the averages of concentrations of different serological factors at similar hours in all groups were analyzed by one-way ANOVA, and the least significant difference (LSD) test was used to find differences. Repeated Measures ANOVA was used to evaluate the changing patterns of all evaluated parameters in each group using SPSS software (SPSS for Windows, version 20, SPSS Inc, Chicago, IL, USA). The level of significance was set at P<0.05.

Results

Alterations in circulating metabolic biomarkers following bolus intravenous glucose administration are presented in Figs. 1 to 4.

The base line levels of NEFA and BHBA at hour zero in early and mid lactation and close-up dry cows were significantly higher than other groups. The significant decreasing pattern of NEFA and BHBA were seen in early and mid lactation and close-up dry cows after glucose administration (P<0.05). There were no significant changing patterns in NEFA and BHBA concentrations after glucose infusion in late lactation and far-off dry cows (P>0.05; Fig. 1).

Rapid and significantly increasing patterns of glucose and insulin concentrations were seen in early and mid lactation and close-up dry cows after glucose administration (P<0.05). In early lactation and close-up dry cows, insulin was remained at high concentrations at hour 4. Despite the increased levels of insulin in early and mid lactation and close-up dry cows, glucose concentrations did not decrease near to base line levels at the 4th hour. Finally, serum concentration of glucose reached base values at the 4th hour in late lactation and far-off dry groups (Fig. 2).

There were no significant changing patterns in lipid profile (cholesterol, TG, HDL, LDL and VLDL) after glucose administration (P>0.05; Figs. 3 and 4). Serum concentrations of cholesterol in mid and late lactation were significantly higher than other groups at all sampling times (P<0.05; Fig. 3). Mid lactation cows had the highest concentrations of LDL in comparison to other animals (P<0.05; Fig. 4). There were no significant differences between each TG, HDL and VLDL values before and after glucose administration in any studied group (P>0.05; Fig. 3 and 4).

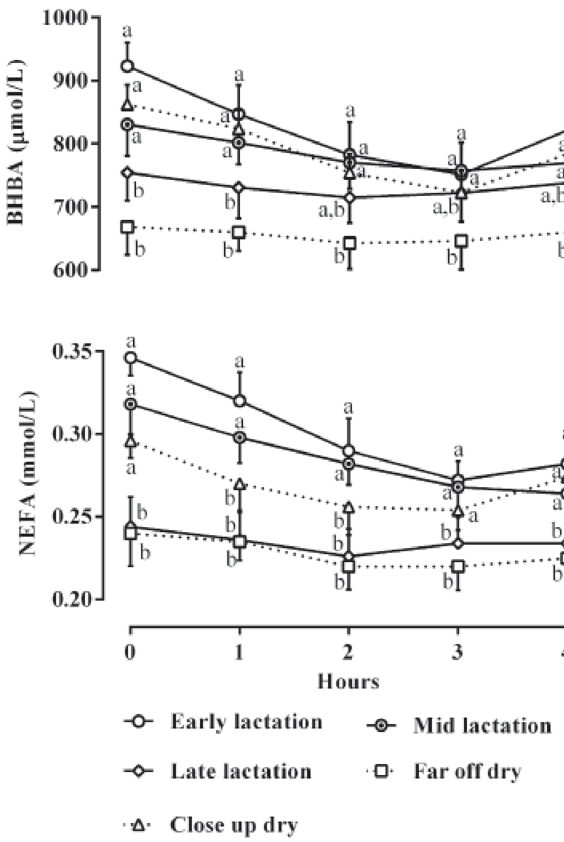

Fig. 1. The changing patterns of BHBA and NEFA following bolus intravenous glucose administration (dextrose 50 %, at 500 mg/kg, 10 mL/kg/hour) in various physiological states of high producing Holstein dairy cows. a,b,c - different letters indicate significant differences in each group at similar times (P<0.05).

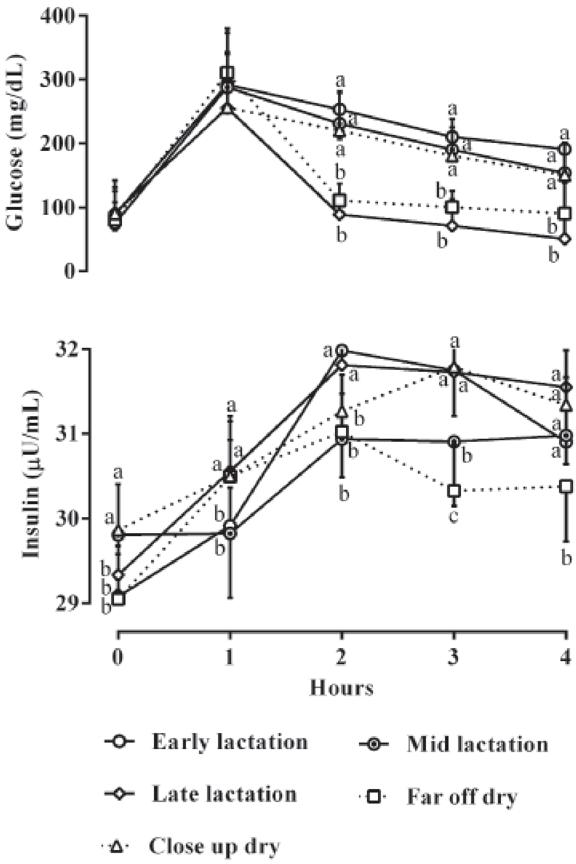

Fig. 2. The changing patterns of glucose and insulin following bolus intravenous glucose administration (dextrose 50 %, at 500 mg/kg, 10 mL/kg/hour) in various physiological states of high producing Holstein dairy cows. a,b,c - different letters indicate significant differences in each group at similar times (P<0.05).

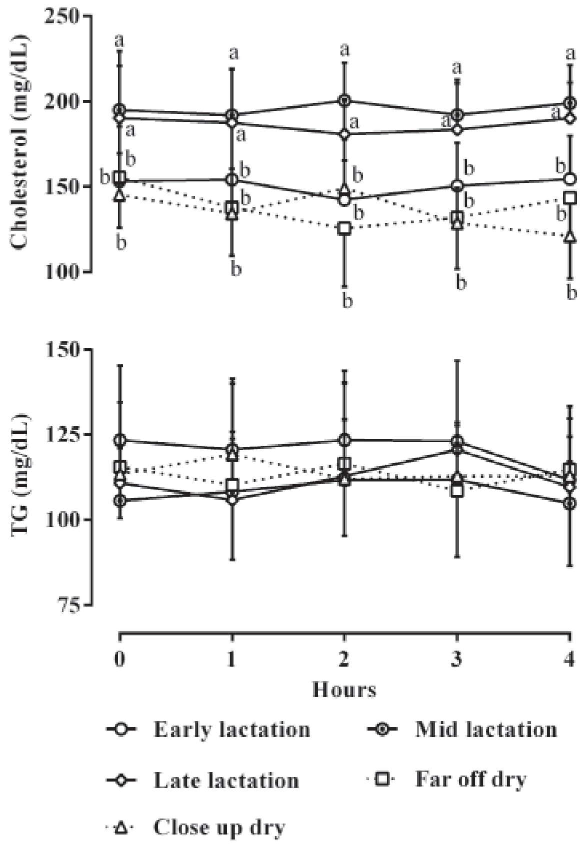

Fig. 3. The changing patterns of cholesterol and TG following bolus intravenous glucose administration (dextrose 50 %, at 500 mg/kg, 10 mL/kg/hour) in various physiological states of high producing Holstein dairy cows. a,b,c - different letters indicate significant differences in each group at similar times (P<0.05).

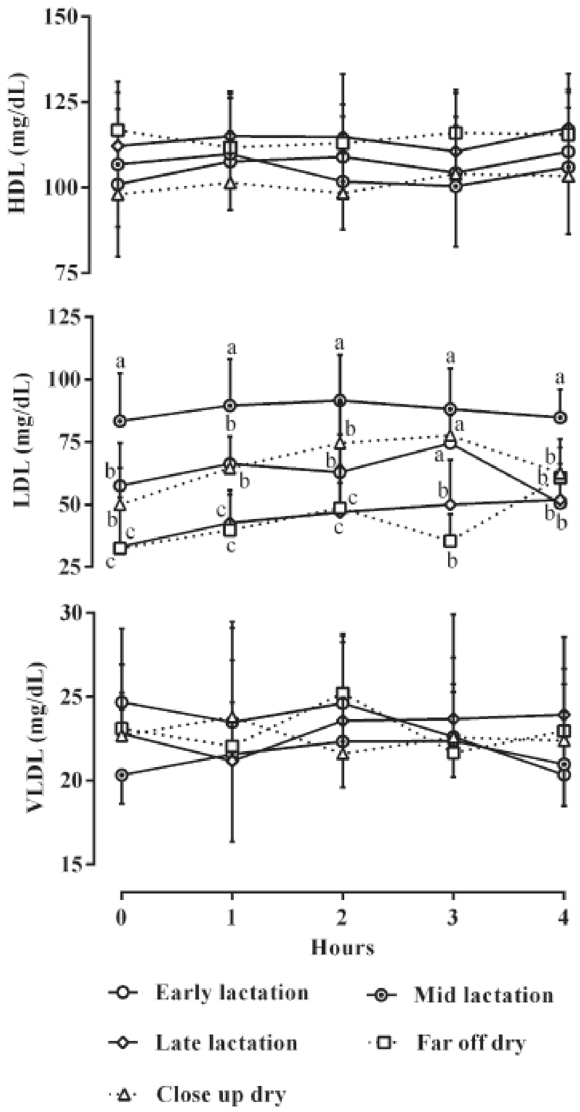

Fig. 4. The changing patterns of HDL, LDL and VLDL following bolus intravenous glucose administration (dextrose 50 %, at 500 mg/kg, 10 mL/kg/hour) in various physiological states of high producing Holstein dairy cows. a,b,c - different letters indicate significant differences in each group at similar times (P<0.05).

Discussion

In ruminants volatile fatty acids from the gastrointestinal tract are the major energy source, rather than direct sources of glucose. Thus, insulin plays a slightly different role in ruminants vs. non-ruminants, although many aspects of insulin metabolism are the same (DE KOSTER and OPSOMER, 2013). In the late lactation and far-off dry cows in this study, serum glucose concentrations were decreased near to baseline levels at the 4th hour after glucose infusion, which indicates the effect of insulin on clearance of glucose. Since the high levels of glucose were cleared after increasing insulin values, it may be concluded that there was no insulin resistance in these cows. However, in early and mid lactation and close-up dry cows, the clearance of glucose was not parallel to increased insulin concentration, which indicates insulin resistance. The highest degree of insulin resistance was seen in the early lactation group (Fig. 2). Insulin resistance involves changes in sensitivity (the amount of hormone required to illicit a response) or responsiveness (the maximum response to a hormone) (KAHN, 1978).

Cows during the late lactation and early far-off dry periods experience relatively low metabolic demands as fetal growth is just beginning to accelerate and the cows are no longer lactating. Therefore, the cows are not in a negative energy balance and insulin resistance does not occur in these periods (BELL, 1995). HOLTENIUS et al. (2003) recently reported that cows fed higher energy levels during the dry period had a greater degree of insulin resistance before and after calving, which induced higher plasma NEFA concentrations, compared to those in cows fed below requirements. HAUGUEL et al. (1987) showed that, in rabbits, the insulin sensitivity of insulin-sensitive tissues decreased during late pregnancy. This may be associated with a decrease in the number of insulin receptors during late pregnancy (VERNON et al., 1981). It was reported that insulin clearance rates had a tendency to decrease during the postpartum period (BOSSAERT et al., 2008). The reduced clearance of glucose observed at post partum was due to a greater degree of insulin resistance, which gave rise to more pronounced net lipolysis from the adipocytes (PIRES et al., 2007). These qualitative changes in metabolism occurring during lactation are necessary to support a homeostatic state (HAYIRLI, 2006). After calving, the initiation of milk synthesis and rapidly increasing milk production greatly increases the demand for glucose for milk lactose synthesis, at a time when feed intake has not reached its maximum (DE KOSTER and OPSOMER, 2013).

NEFA levels were higher in early and mid lactation and close-up dry cows before glucose infusion and for all the different times following glucose infusion (Fig. 1). These results indicate the activation of lipid mobilization that represents another metabolic mechanism of adaptation to these periods (PICCIONE et al., 2012). In fact, it is well known that the low insulin concentration and reduced insulin sensitivity of the tissues around parturition increases lipid mobilization and induces further rises in plasma NEFA concentrations (HAYIRLI, 2006). The early decrease of NEFA values after glucose administration demonstrates that the lipid mobilization of fatty acids is reduced in positive energetic balance conditions.

The plasma BHBA profile followed the same pattern as the NEFA concentration (Fig. 1). According to GRUMMER et al. (2004), the increase in plasma NEFA concentration led to an increase in ketogenesis by hepatocytes. BHBA levels were higher in early and mid lactation and close-up dry cows before glucose infusion, and for all the different times following glucose infusion in these groups (Fig. 1). The early decrease of BHBA levels in response to glucose administration demonstrates that, following glucose infusion, the production of ketone bodies was reduced.

Serum concentrations of lipid profile did not change after bolus intravenous glucose administration in any of the animals studied (Figs. 3 and 4). As the concentration of NEFAs in the blood increases around calving or in early lactation, more NEFAs are taken up by the liver (EMERY et al., 1992). Once taken up by the liver, NEFAs can be completely oxidized into carbon dioxide to provide energy for the liver, partially oxidized to produce ketone bodies that are released into the blood and serve as fuels for other tissues, or reconverted to storage fat (TGs). Ruminants have an inherently low capacity for synthesis and secretion of VLDL to export TG from the liver (PULLEN et al., 1989), but a similar capacity to reconvert NEFAs back to TG (GRAULET et al., 1998). Moreover, the rate of production of TGs in the liver is increased at the time of calving (GRUM et al., 1996). Consequently, cows fed typical diets during the dry period and transition period have increased concentrations of TG in the liver 1 day after calving (GRUM et al., 1996). If NEFA uptake by the liver becomes excessive, fatty liver may develop. Negative energy balance and carbohydrate insufficiency in the liver after calving leads to increased production of ketone bodies, which can result in ketosis (DE KOSTER and OPSOMER, 2013).

Total cholesterol was increased during mild lactation (Fig. 3). This was probably because, during the puerperal period, there is an increase in demand for the regulatory mechanism responsible for all the processes involved with milking (KRAJNICAKOVA et al., 2003). For this purpose, characteristic changes in lipid metabolism were found during pregnancy and lactation in most mammals (ROCHE et al., 2009). Endocrine profiles change, and lipolysis and lipogenesis are regulated to increase lipid reserves during pregnancy, and, subsequently, these reserves are utilized following parturition and the initiation of lactation (ROCHE et al., 2009). Similar results were found by other researchers, demonstrating that concentrations of total lipid increased atparturition, despite the kind of fed administered (DOUGLAS et al., 2004).

Conclusion

The results of this study demonstrated that bolus intravenous glucose infusion can influence metabolism in high producing Holstein dairy cows. The changing patterns of circulating metabolic profile indicated that glucose is an important direct controller of metabolic interactions and responses in dairy cows in various physiological states. Finally, bolus intravenous glucose administration can be used as a regimen to prevent and control metabolic disorders in dairy cows.

Conflict of interest statement

The authors have declared no conflicts of interest.

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SAŽETAK

Zadovoljenje potreba za energijom, s posebnim naglaskom na opskrbu glukozom, može spriječiti metaboličke poremećaje kod visokomliječnih krava. Stoga se pretpostavlja da intravenska primjena bolusa glukoze može mijenjati koncentracije cirkulirajućih metaboličkih biomarkera s ciljem sprječavanja i kontrole metaboličkih poremećaja u mliječnih krava. Dvadeset pet visokomliječnih krava holštajnske pasmine, višerotki, podijeljeno je u 5 jednakih skupina prema ranoj, srednjoj i kasnoj laktaciji te prema vremenu (kratko ili dugo) do zasušenja. Sve krave su primile 50 %-tnu dekstrozu, intravenski 500 mg/kg, 10 mL/kg/sat. Uzorci krvi prikupljeni su od svih životinja prije, zatim 1, 2, 3 i 4 sata nakon infuzije 50 %-tne dekstroze. U serumu su određene koncentracije glukoze, inzulina, neesterificiranih masnih kiselina, β-hidroksimaslačne kiseline, kolesterola, triglicerida, te lipoproteina visoke, niske i vrlo niske gustoće. Signifikantno ($P<0,05$) smanjenje neesterificiranih masnih kiselina i beta-hidroksimaslačne kiseline ustanovljeno je nakon primjene glukoze kod krava u ranoj i srednjoj laktaciji te kod krava s kratkim vremenom do zasušenja. Brzo i značajno povećanje koncentracija glukoze i inzulina opaženo je nakon primjene glukoze kod krava u ranoj i srednjoj laktaciji, te kod krava s kratkim vremenom do zasušenja. Kod krava u ranoj laktaciji i kratkim vremenom do zasušenja, inzulin je 4 sata ostao na visokim koncentracijama. Usprkos povećanim razinama inzulina kod krava u ranoj i srednjoj laktaciji te kratkim vremenom do zasušenja, koncentracije glukoze nisu smanjene do bazne razine 4. sata. Nakon primjene glukoze, nije bilo signifikantne ($P>0,05$) promjene u lipidnom profilu. Rezultati ovog istraživanja pokazali su da intravenska infuzija bolusa glukoze može utjecati na metabolizam visokoproizvodnih krava holštajnske pasmine. Promjenjljivost profila cirkulirajućih metabolita ukazuje da je glukoza važan izravan upravljač metaboličkih interakcija, te da je odgovorna za različita fiziološka stanja u mliječnih krava.

Ključne riječi: glukoza, energetske potrebe, potpora metabolizmu, holštajnske krave