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RESEARCH ARTICLE

Estimation of Insulin Resistance in Healthy and Ketotic Cows during an Intravenous Glucose Tolerance Test

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The objective of this study was to determine insulin resistance in healthy (n=8) and ketotic (n=7) dairy cows based on the difference between basal and dynamic changes in glucose, insulin, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB) concentrations, and on the determination of their mutual relationship and relationship with insulin resistance indices (RQUICKI and RQUICKI-BHB), after intravenous glucose tolerance test (IVGTT). The RQUICKI index showed a significant linear correlation ($P<0.05$) with basal insulin, glucose, NEFA and BHB levels. However, RQUICKI-BHB index values exhibited a negative correlation ($P<0.01$) with basal NEFA and BHB values and their clearance rates, as well as with glucose clearance rate, and a positive correlation ($P<0.05$) with basal insulin values as well as with insulin and glucose responses during IVGTT. The correlation between basal values of these parameters and the values measured or calculated during IVGTT is the result of RQUICKI-BHB values, as the exclusion of RQUICKI-BHB leads to loss of the statistical significance of the correlations between basal and dynamic values. Insulin resistance in ketotic cows is characterized by decreased insulin response to glucose and increased insulin resistance of the tissue, their correlation being the result of the RQUICKI-BHB index value.

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INTRODUCTION

To maintain the health of early lactating dairy cows, it is critical to ensure the optimal glucose supply to the mammary gland and liver. Metabolic changes in early lactation are accompanied with ketosis in dairy cows (Youssef and El-Ashker, 2017). Hypoglycemia is the first clinical sign of the metabolic disorder associated with primary ketosis in dairy cows during early lactation. It induces serious metabolic changes, leading to mobilization of lipid reserves, higher ketone body synthesis and lipogenesis in the liver (Hayirli, 2006; Piccione *et al.*, 2012). Postpartum high-yielding dairy cows exhibit insulin resistance to assist in directing nutrients from insulin sensitive tissues, while the increase in glucose clearance rate observed during postpartum, as compared with the prepartum period, is most likely the result of increased glucose uptake by the mammary gland (Holtenius *et al.*, 2003; Bossaert *et al.*, 2008; Asif *et al.*, 2015). Insulin resistance involves a decrease in insulin responsiveness, i.e. pancreatic beta-cell function, and/or insulin sensitivity, i.e. glucose entry into tissues through the action of insulin. Insulin resistance during the periparturient period is needed to the udder, as an organ in which glucose utilization is not insulin-dependent, given enough nutrients and energy to initiate lactation (Hayirli, 2006; De Koster and Opsomer, 2013). Malnutrition causes low basal insulin and basal glucose concentrations with

lower insulin response to glucose administration (Hayirli, 2006). High concentrations of BHB decrease glucose concentrations, and do not increase insulin concentrations postpartum (Zarrin *et al*., 2017).

An intravenous glucose tolerance test (IVGTT) is conducted to evaluate the insulin synthesis and secretion by the endocrine pancreas in ruminants (Djokovic *et al.*, 2009; De Koster *et al.*, 2017). Based on the higher glucose concentrations and lower clearance rates in early lactation, we conclude that insulin resistance exists during early lactation (Chalmeh *et al.*, 2015). Another way to test insulin resistance is to determine the RQUICKI and RQUICKI-BHB insulin resistance indices, which depend on the basal insulin, glucose, fatty acid and ketone concentrations, and show a negative correlation with the glucose concentration and intensity of change during IVGTT (Holtenius and Holtenius, 2007; Balogh *et al.*, 2008). RQUICKI values are lower in ketotic cows (Xu *et al.*, 2014) and indicate a negative correlation with BHB values (Cincović *et al.*, 2014). Consequently, they are important for the metabolic prediction in early lactation (Cincović *et al.*, 2017).

This research aimed to determine insulin resistance in healthy and ketotic dairy cows based on the difference between basal and dynamic changes in glucose, insulin, NEFA and BHB concentrations and on the determination of their mutual relationship and relationship with insulin resistance indices (RQUICKI and RQUICKI-BHB).

MATERIALS AND METHODS

Animals: The experiment involved eight healthy and seven ketotic Holstein-Friesian dairy cows from day 7 to day 14 of early postpartum lactation. Ketosis was diagnosed by observing the clinical symptoms (ruminal atony, reduced appetite, behavioral changes), including high concentrations of β-hydroxybutyrate in the blood (>2.6 mmol/L) (Oetzel, 2004) and ketone bodies in the urine. The cows had similar body weights (560-580 kg); they were aged 4-6 years, and had 3 lactations on average with a mean 305-day milk yield of 7850±550 L during the previous lactation. The cows were fed a meal containing 7 kg alfalfa hay, 20 kg corn silage (30% dry matter, DM) and 5 kg concentrate (18% crude protein, CP) which met their energy requirements in early lactation. The chemical characteristics of the diet were as follows: 87.15 MJ NEL; crude protein 13.58% DM; rumen undegradable protein 35.91% of crude protein; fat 3.09% DM, fiber 23.26% DM.

Intravenous glucose tolerance test and insulin resistance: The cows were tested at 9 a.m. about 3 h after feeding. Each animal received 500 ml of 50% glucose solution infused for 5 minutes into the jugular vein. Blood samples were taken from the opposite jugular vein prior to infusion (0) and at 5, 10, 30, and 60 min after infusion. The area under the curve (AUC) for glucose and insulin was calculated by trapezoidal rule when baseline level is Y=0 (Cardoso *et al.*, 2011). *AUCtotal* represents AUC from 0 to 60 minutes of test. *AUCmax* is the area under the curve from 0 to 5 minutes for glucose, or from 0 to 10 minutes for insulin (time when maximal concentration of insulin or glucose was achieved). Clearance % min was calculated as follows [100-(end concentration/start concentration) x 100)]/end time–start time; for glucose (end concentration at 60 min, start 5 min), NEFA and BHB (end concentration at 10 min and start at 0 min). Insulin resistance indices were calculated by standard methodology (Holtenius and Holtenius, 2007; Balogh *et al.*, 2008): RQUICKI = 1/[log (conc.*t*0 glucose mg/dL) + log (conc.*t*0 insulin mmol/L)+log (conc.*t*0 NEFA mmol/L)]; RQUICKI-BHB = 1/[log (conc.*t*0 glucose mg/dL) + log (conc.*t*0 insulin mmol/L)+log (conc.*t*0 NEFA mmol/L) + log (conc.*t0* BHB mmol/L)].

Biochemical analysis: Blood samples were allowed to clot for 3 hours at 4°C prior to centrifugation (1500 g, 10 min). Then, the serum was carefully collected and stored at -20°C pending analysis. Blood samples were collected in vacum tubes containing fluoride, which were immediately subjected to centrifugation as described. Serum insulin levels were determined by ELISA (Cusabio, Ch) using a Rayto reader (Ch). Measurement of glucose (glucose oxidase test), NEFA (acyl-CoA sintetase test) and BHB (3-hydroxybutyrate dehydrogenase test) was performed by standard colorimetric method using a Randox (UK) kit and a Rayto spectrophotometer (Ch).

Statistical analysis: The influence of time after IVGTT on glucose, insulin, NEFA and BHB was analyzed by an ANOVA analysis with post hoc LSD test. Differences in AUCtotal, AUCmax and clearance % min, RQUICKI and RQUICKI-BHB between healthy and ketotic cows were determined by Student t-test. Correlation between basal (glucose basal, insulin basal, NEFA basal, BHB basal), maximal (glucose max, insulin max) or minimal (NEFAmin, BHBmin) concentrations during IVGTT, AUCtotal, AUCmax, clearance, RQUICKI and RQUICKI-BHB was determined by the Pearson correlation coefficient and presented as a correlation matrix. The partial correlation method was used to determine whether the strength of the linear correlation between basal and dynamic values of the metabolites was dependent on the values of insulin resistance indices. The statistical software Statgraphic Centurion (Statpoint Technologies Inc. Warrenton, Va, Virginia, USA) was used for these purposes.

RESULTS

Blood glucose concentration in healthy and ketotic cow during IVGTT are presented in Fig. 1. Ketotic cows were found to have significantly ($P<0.01$) lower initial blood glucose values compared to healthy animals (2.17±0.16: 3.30±0.24 mmol/L). After the intravenous administration of glucose solution, the glycemic levels increased significantly ($P<0.05$) in both ketotic and healthy cows at 5, 10 and 30 minutes of the experiment, reaching their peak at 5 minutes and slowly declining thereafter. Mean blood glucose levels were significantly ($P<0.01$) lower in ketotic cows than in healthy animals throughout the experiment.

Blood insulin concentration in healthy and ketotic cow during IVGTT are presented in Fig. 2. The mean initial levels of insulin in the blood were decreased but no significant differences were observed in healthy cows (203.7±46.5 pmol/L) compared to ketotic cows

(224.2±26.7 pmol/L). Insulin concentration increased significantly (P<0.05) 10 minutes after glucose intravenous infusion in both groups of cows. Specifically, blood insulin levels increased to 881.2±144.7 pmol/L at 10 minutes in healthy cows compared with 370.0±92.4 pmol/L in ketotic cows (P<0.01). Thereafter, blood insulin concentrations decreased in both groups of cows.

Blood BHB concentration in healthy and ketotic cow during IVGTT are presented in Fig. 3. Blood BHB levels were initially high in ketotic cows in comparison to healthy cows (2.57±0.45 vs 0.51±0.12 mmol/L; P<0.01). Minimal concentrations of BHB at 10 min after glucose infusion were higher in ketotic cows than in healthy animals (P<0.01), with the difference still maintained in the same way.

Blood NEFA concentration in healthy and ketotic cow during IVGTT are presented in Fig. 4. Basal NEFA concentrations were higher in ketotic cows than in healthy animals (0.60±0.14 vs 0.50±0.09 mmol/L; P<0.05). Blood NEFA levels significantly dropped 10 minutes after intravenous glucose infusion (P<0.01), with the difference between the groups which were managed similarly.

Parameters calculated from IVGTT curve (AUC and clerance) and indices of insulin sensitivity (RQUICKI and RQUICKI-BHB) are presented in Table 1. Ketotic cows showed lower AUC total and lower AUC max both for glucose and insulin in comparison with healthy cows (P<0.01). Also, ketotic cows had a lower clearance rate of glucose, NEFA and BHB compared to healthy cows (P<0.01). The difference in RQUICKI value was not observed between healthy and ketotic cows (P>0.05). However, RQUICKI-BHB value was significantly lower in ketotic cows (P<0.01).

Correlation coefficients between concentrations and AUCs obtained during IVGTT and indexes of insulin resistance in healthy and ketotic cows are presented in Table 2. Basal insulin concentration is negatively correlated (P<0.05) with BHB concentration. Basal glucose concentration is negatively correlated with NEFA (P>0.05) and BHB (P<0.01) values. No significant correlation (P>0.05) was determined between insulin and glucose basal concentrations. Basal insulin value is positively correlated (P<0.01) with maximum insulin values, AUCmax and AUCtotal for insulin during IVGTT. Basal glucose concentration shows a positive correlation (P<0.05) with glucose max and AUC max for glucose. NEFA, BHB and glucose clearance rates increased with increasing responses of insulin and glucose during IVGTT (P<0.01). Basal NEFA and BHB values are negatively correlated (P<0.05) with insulin and glucose responses during IVGTT. Interestingly, basal BHB and NEFA values are negatively correlated (P<0.01) with the clearance rate of these substances during IVGTT. The RQUICKI index shows a significant linear correlation (P<0.05) with basal insulin, glucose, NEFA and BHB concentrations, but not with any of dynamic changes during IVGTT. However, RQUICKI-BHB index value is negatively correlated (P<0.01) with NEFA and BHB values and their clearance rates, as well as with glucose clearance. Also, it is positively correlated (P<0.05) with basal insulin values, as well as with insulin and glucose responses during IVGTT.

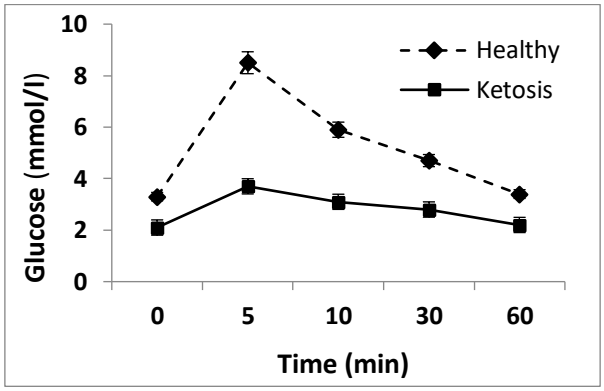

Fig. 1: Changes in blood glucose levels in healthy and ketotic cows after intravenous glucose administration.

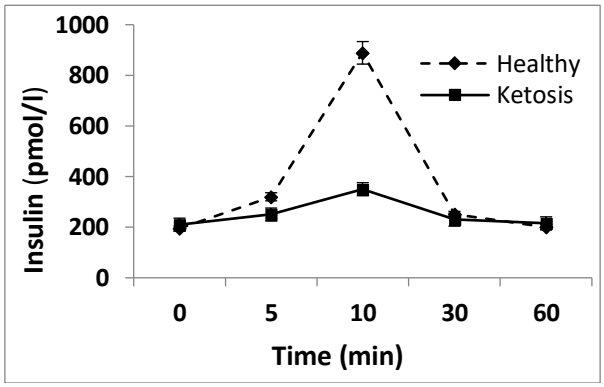

Fig. 2: Changes in blood insulin levels in healthy and ketotic cows after intravenous glucose administration.

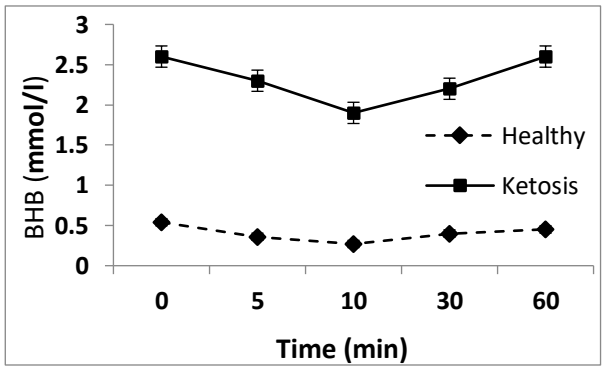

Fig. 3: Changes in blood BHB levels in healthy and ketotic cows after intravenous glucose administration.

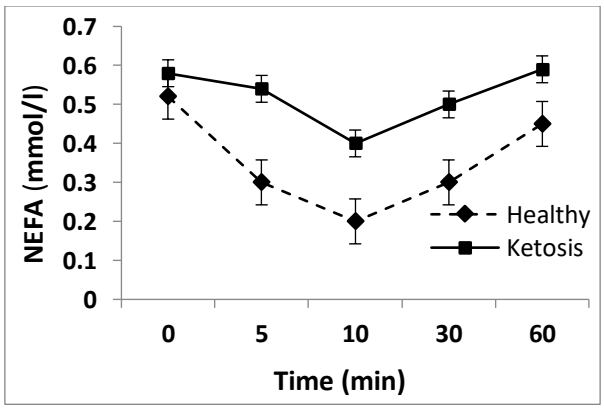

Fig. 4: Changes in blood NEFA levels in healthy and ketotic cows after intravenous glucose administration.

Table 1: Parameters calculated from IVGTT curve and RQUICKI and RQUICKI-BHB indices of insulin sensitivity

	Healthy cows	Ketotic cows	P
Insulin AUCtotal	22450.5±2150.3	15125.2±1649.1	<0.01
Insulin AUCmax	15732.1±1596.5	8910.2±707.7	<0.01
Glucose AUCtotal	293.8±33.5	165.5±20.1	<0.01
Glucose AUCmax	65.9±7.83	40.3±3.92	<0.01
Glucose clearance %/min	1.08±0.14	0.71±0.11	<0.01
NEFA clearance %min	6.12±1.24	3.42±0.93	<0.01
BHB clearance %min	4.1±0.54	2.3±0.21	<0.01
RQUICKI	0.34±0.021	0.36±0.022	ns
RQUICKI-BHB	0.38±0.035	0.31±0.031	<0.01

Legend: ns-non significant.

Results show that the correlation between basal values of the parameters at 0 minutes and the values measured or calculated during IVGTT is largely due to the value of RQUICKI-BHB at 0 minutes, since the exclusion of RQUICKI-BHB results in a statistically insignificant correlation between basal and dynamic values (Table 3).

DISCUSSION

Basically, the IVGTT evaluates the primary function of beta cells to synthesize and release insulin. The pattern of response of plasma glucose and insulin to IVGTT in the present study was similar to the pattern reported in other studies (Holtenius *et al.*, 2003; Hayirli, 2006; Djokovic *et al.*, 2007, 2009). Parameters from IVGTT showed good correlation with HEIC parameters (De Koster *et al*., 2016), but Mann *et al.* (2016) suggest that correlation between insulin resistance indices and IVGTT parameters are generally poor.

Glucose disappearance after IVGTT is the result of glucose utilization by peripheral tissues, absorption from the intestine, glucose production in the liver and excretion through the kidney. Glucose utilization by the udder is significantly increased in early lactation (Holtenius *et al.*, 2003), and the degree of glucose utilization from the circulation is dependent on milk yield. Although hepatic gluconeogenesis is increased in lactating cows (Ingvartsen, 2006), high insulin concentrations during IVGTT decreases gluconeogenesis in liver cells (Brockman and Laarveld, 1986). In this study, glucose clearance suggests that blood glucose utilization by peripheral tissues is higher in healthy cows than in ketotic ones. The increased rate of glucose disappearance from the plasma is induced by increased insulinemia.

In lactating dairy cows, insulin resistance occasionally means depression of pancreatic insulin secretion (Holtenius *et al.,* 2003). The present experiment showed that healthy cows had larger insulin area under the curve, most likely resulting from increased insulin secretion. The significant increase in blood insulin levels relative to initial values in ketotic and healthy cows 5 and 10 minutes after IVGTT serves as confirmation of the ability of glucose to affect the production and release of insulin from endocrine pancreatic beta cells. Moreover, blood insulin levels in ketotic cows were significantly lower than in healthy animals, at 10, 30 and 60 minutes of the testing. Therefore, the low release of insulin in ketotic cows is likely due to the low pancreatic capacity for insulin secretion that develops during the days or weeks of malnutrition-induced hypoglycemia which is generally accompanied by ketosis. Prolonged malnutrition causes a reduction in the number and size of islets, potentially leading to a decrease in insulin secretion (Tse *et al.*, 1998). The results showed the preserved function (relative insufficiency) of endocrine pancreatic beta cells in ketotic cows. Similar results were reported elsewhere (Sakai *et al.*, 1993; 1996; Holtenius *et al.*, 2003; Djokovic *et al.*, 2007). Insulin and lipid metabolites showed an inverse relation (Bossaert *et al.*, 2008). Insulin has an antiketogenic effect by inhibiting lipolysis, stimulating lipogenesis, and by enhancing ketone body utilization in the peripheral tissue (Brockman and Laarveld, 1986). Moreover, insulin increases hepatic enzyme activity for re-esterification of NEFA into triacylglycerol (Emmison *et al.*, 1992), and it reduces the hepatic mitochondrial oxidation of NEFA by the insulin-induced decrease in carnitine palmitoyltransferase-1 (CPT-1) activity whereby the mitochondrial uptake of NEFA is diminished (Zammit, 1996).

Table 2: Correlation coefficients between concentrations and AUCs obtained during IVGTT and indexes of insulin resistance in healthy and ketotic cows

	Insulin max	Insulin AUC max	Insulin AUC total	Glucose basal	Glucose max	Glucose AUC max	Glucose AUC total	Glucose clearance	NEFA basal	NEFA min	NEFA clearance %	BHB basal	BHB min	BHB clearance %	RQUICKI	RQUICKI-BHB
Insulin basal	0.69**	0.58*	0.63**	0.43^{NS}	0.49 ^{ns}	-0.39^{ns}	0.38^{ns}	-0.26^{ns}	-0.42^{ns}	-0.32^{ns}	-0.19^{ns}	-0.55*	-0.27^{ns}	0.31^{ns}	0.52*	0.57*
Insulin max	1	0.64**	0.58*	0.59*	0.63**	0.71**	0.34^{ns}	0.72**	-0.57*	-0.63**	0.68**	-0.76**	-0.69**	0.62**	0.35^{ns}	0.52*
Insulin AUC max		1	0.71*	0.52*	0.54*	0.61**	0.52*	0.63**	-0.59*	-0.59*	0.65**	-0.65**	-0.72**	0.59*	0.28^{na}	0.62**
Insulin AUC total			1	0.56^{ns}	0.42^{ns}	0.53*	0.46^{ns}	0.71**	-0.52*	-0.32^{ns}	0.52*	-0.72**	-0.63**	0.43 ^{ns}	0.33^{ns}	0.43^{ns}
Glucose basal				1	0.51*	0.63**	0.49^{ns}	0.32^{ns}	-0.61**	-0.33^{ns}	0.36^{ns}	-0.62**	0.32^{ns}	0.28^{ns}	0.59*	-0.38^{ns}
Glucose max					1	0.71**	0.59*	0.62**	-0.63**	-0.7**	0.52*	-0.67**	-0.59*	0.62**	0.42^{ns}	0.59*
Glucose AUC max						1	0.64**	0.32^{ns}	-0.56*	-0.62**	0.6**	-0.52**	-0.53*	0.57*	0.26^{ns}	0.67**
Glucose AUC total							1	0.26^{ns}	-0.33^{ns}	-0.26^{ns}	0.36^{ns}	-0.54*	-0.59*	-0.26^{ns}	0.43^{ns}	0.44^{ns}
Glucose clearance								1	-0.66**	0.44^{ns}	0.68**	0.52*	0.37^{ns}	0.69**	0.47^{ns}	-0.72**
NEFA basal									1	0.33^{ns}	-0.53*	0.51*	0.26^{ns}	-0.63**	-0.65**	0.48^{ns}
NEFA min										1	0.22^{ns}	0.51**	0.35^{ns}	0.19^{ns}	0.29^{ns}	-0.59*
NEFA clearance %											1	-0.54*	0.22^{ns}	0.76**	0.3^{ns}	-0.67**
BHB basal												1	0.54*	-0.64**	-0.64**	-0.71**
BHB min													1	0.29^{ns}	0.39^{ns}	-0.69**
BHB clearance %														1	-0.29^{ns}	-0.73**
RQUICKI															1	-0.44^{ns}

Legend: Significant correlations are marked with asterisks *(P<0.05) and **(P<0.01); ^{ns}-non significant, max/min- maximal/minimal concentration during IVGTT, AUC total -area under curve from start to end of IVGTT, AUCmax-area under curve from start to time when maximal concentration is achieved, clearance % - change of concentration % min, RQUICKI/RQUICKI-BHB - Revised Quantitative Insulin Sensitivity Check Index / including BHB.

Table 3: Partial correlation of basal glucose, insulin, NEFA and BHB with dynamic change during IVGTT after exclusion of RQUICKI-BHB index

		Insulin basal	Glucose basal	NEFA basal	BHB basal
Insulin	No control	0.69	0.59	-0.57	-0.76
max	RQUICKI-BHB	0.56	0.37$^{\Delta}$	-0.42$^{\Delta}$	-0.65
Insulin	No control	0.58	0.52	-0.59	-0.65
AUC max	RQUICKI-BHB	0.35$^{\Delta}$	0.39$^{\Delta}$	-0.42$^{\Delta}$	-0.38$^{\Delta}$
Insulin	No control	0.63	/	-0.52	-0.72
AUC total	RQUICKI-BHB	0.45$^{\Delta}$		-0.39$^{\Delta}$	-0.65
Glucose	No control	/	0.51	-0.63	-0.67
max	RQUICKI-BHB		0.38$^{\Delta}$	-0.49$^{\Delta}$	-0.44$^{\Delta}$
Glucose	No control	/	0.63	-0.56	-0.52
AUC max	RQUICKI-BHB		0.54	-0.36$^{\Delta}$	0.09$^{\Delta}$
Glucose	No control	/	/	/	-0.54
AUC total	RQUICKI-BHB				-0.36$^{\Delta}$
Glucose	No control	/	/	-0.66	0.52
clearance	RQUICKI-BHB			-0.51	0.02$^{\Delta}$
NEFA	No control	/	-0.61	/	/
basal	RQUICKI-BHB		-0.55		
NEFA	No control	/	/	/	0.51
min	RQUICKI-BHB				0.16$^{\Delta}$
NEFA	No control	/	/	-0.53	-0.54
clearance %	RQUICKI-BHB			-0.32$^{\Delta}$	-0.12$^{\Delta}$
BHB	No control	-0.55	-0.62	0.51	/
basal	RQUICKI-BHB	-0.25$^{\Delta}$	-0.54	0.27$^{\Delta}$	
BHB	No control	/	/	/	0.54
min	RQUICKI-BHB				0.09$^{\Delta}$
BHB	No control	/	/	-0.63	-0.64
clearance %	RQUICKI-BHB			-0.46$^{\Delta}$	-0.25$^{\Delta}$

$^{\Delta}$ - absence of linear correlation after exclusion of RQUICKI-BHB control.

NEFA and BHB generally compromise insulin and glucose response. NEFA not only induces a defect in several pathways of insulin action, but it can also lead to impaired pancreatic insulin production. Bossaert *et al.* (2008) reported that plasma NEFA was negatively correlated with insulin AUC and peak levels, without affecting glucose parameters in postpartal cows. Pires *et al.,* (2007b) found that elevated triglyceride, and more specifically, NEFA levels intensify insulin resistance that could be decreased by the administration of nicotinic acid. Akbari *et al.* (2015) found that experimental hyperlipidemia with high NEFA concentration induced insulin resistance in sheep. A marked decrease in the insulin secretory capacity of beta cells and a decline in insulin responsiveness in ketotic cows were reported in several studies (Sakai *et al.*, 1993, 1996; Djoković *et al.*, 2007, 2009), which is in agreement with our results. Hayirli (2006) reported that elevated NEFA concentrations inhibit the insulin-stimulated glucose utilization by tissues, decrease the number of GLUT 4, and disturb intracellular insulin signaling pathways in the liver and peripheral tissues.

Insulin resistance is a generic term that can be assessed by insulin responsiveness (insulin response to glucose), insulin sensitivity (tissue responsiveness to insulin), or both (Sano *et al.*, 1991; Hayirli, 2006; Balogh *et al.*, 2008). In ketosis, insulin resistance can be evaluated by both processes. This is supported by the fact that ketosis therapy is more efficient after glucose and insulin administration than after glucose administration only (Sakai *et al*., 1993).

Conclusions: Depression in basal glucose concentration, insulin and glucose release and glucose clearance, higher lipid mobilization and ketogenesis are signs of insulin resistance in ruminants caused by malnutrition, which is accompanied by ketosis. The insulin resistance in ketotic cows involves a reduced insulin response to glucose and an increased insulin resistance in tissues, and their correlation is the result RQUICKI-BHB values.

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Authors contribution: RDJ and VD carried out biochemistry laboratory analysis, participated in the design and drafted the manuscript. MRC conceived of the study, participated in its design and coordination, helped to draft the manuscript and performed statistical analysis. BB and NF participated in design of study and helped to draft the manuscript. BJ and ML carried out the immunoassays. All authors read and approved the final manuscript.

REFERENCES

Akbari H, Dalir Naghadeh B, Asri-Rezaei S, *et al*., 2015. Experimental hyperlipidemia induces insulin resistance in sheep. Domest Anim Endocrinol 53:95-102.

Asif AR, Qadri S, Fu Y, *et al*., 2016. Single nucleotide polymorphisms in DRB1, IGF1 and ILS associated with fecal egg count confers resistance against *Haemonchus contortus* infection in goats. Pak J Agric Sci 53:963-70.

Balogh O, Szepes O, Kovacs K, *et al.,* 2008. Interrelationships of growth hormone Alu I polymorphism, insulin resistance, milk production and reproductive performance in Holstein-Friesian cows. Vet Med 53:604-16.

Bossaert P, Leroy MR, De Vliegher S, *et al*., 2008. Interrelations between glucose-induced insulin response, metabolic indicators, and time of first ovulation in high-yielding dairy cows. J Dairy Sci 91:3363-71.

Brockman RP and Laarveld B, 1986. Hormonal-regulation of metabolism in ruminants-A review. Livest Prod Sci 14:313-34.

Cardoso FC, Sears W, Le Blanc SJ, *et al*., 2011. Technical note: comparison of 3 methods for analyzing areas under the curve for glucose and nonesterified fatty acids concentrations following epinephrine challenge in dairy cows. J Dairy Sci 94:6111-15.

Chalmeh A, Pourjafar M, Nazifi S, *et al*, 2015. Insulin resistance in different physiological states of high producing holstein dairy cows. Acta Sci Vet 43:1255.

Cincović MR, Belić B, Đoković R, *et al*., 2014. Insulin resistance in cow during dry period and early lactation. Contemp Agric 63:98-105.

Cincović M, Kirovski D, Vujanac I, *et al*., 2017. Relationship between the indexes of insulin resistance and metabolic status in dairy cows during early lactation. Acta Vet (Belgrade) 67:57-70.

De Koster JD and Opsomer G, 2013. Insulin resistance in dairy cows. Vet Clin North Am Food Anim Pract 29:299-322.

De Koster J, Hostens M, Hermans K, *et al*., 2016. Validation of different measures of insulin sensitivity of glucose metabolism in dairy cows using the hyperinsulinemic euglycemic clamp test as the gold standard. Domes Anim Endocrinol 57:117-26.

De Koster J, Van Eetvelde M, Hermans K, *et al*., 2017. Short communication: Limitations of glucose tolerance tests in the assessment of peripheral tissue insulin sensitivity during pregnancy and lactation in dairy heifers. J Dairy Sci 100: 2381-87.

Djokovic R, Šamanc H, Nikolic Z, *et al*., 2007. Changes in blood values of glucose, insulin and inorganic phosphorus in healthy and ketotic dairy cows after intravenous infusion of propionate solution. Acta Vet Brno 76:525-32.

Djoković R, Šamanc H, Ilić Z, *et al*., 2009. Changes in blood values of glucose, insulin and inorganic phosphorus in healthy and ketotic dairy cows after intravenous infusion of glucose solution. Acta Vet Brno 78:449-53.

Emmison NV, Zammit A and Agius L, 1992. Triacylglycerol accumulation and secretion in hepatocyte cultures. Effects of insulin, albumin and Triton WR 1339. Biochem J 285:655-60.

Hayirli A, 2006. The role of exogenous insulin in the complex of hepatic lipidosis and ketosis associated with insulin resistance phenomenon in postpartum dairy cattle. Vet Res Commun 30:749-74.

Holtenius K, Agenas S, Delavaud C, *et al*., 2003. Effects of feeding intensity during the dry period. 2. Metabolic and hormonal responses. J Dairy Sci 86:883-91.

Holtenius P and Holtenius K, 2007. A model to estimate insulin sensitivity in dairy cows. Acta Vet Scand 49:29-31.

Ingvartsen KL, 2006. Feeding- and management-related diseases in the transition cow. Physiological adaptations around calving and strategies to reduce feeding-related diseases. Anim Feed Sci Tech 126:175-213.

Mann S, Leal Yepes FA, Duplessis M, *et al*., 2016. Dry period plane of energy: Effects on glucose tolerance in transition dairy cows. J Dairy Sci 99:701-17.

Oetzel GR, 2004. Monitoring and testing dairy herds for metabolic disease. Vet Clin North Am Food Anim Pract 20:651-74.

Piccione G, Messina V, Marafioti S, *et al*., 2012. Changes of some haematochemical parameters in dairy cows during late gestation, post partum, lactation and dry periods. Vet Med Zoot 58:59-64.

Pires JAA, Pescara JB and Grummer RR, 2007. Reduction of plasma NEFA concentration by nicotinic acid enhances the response to insulin in feed-restricted Holstein cows. J Dairy Sci 90:4635-42.

Sakai T, Hamakawa T, Hamakawa M, *et al*., 1993. Therapeutic effects of simultaneous use of glucose and insulin in ketotic cows. J Dairy Sci 76:109-14.

Sakai T, Hamakawa M and Kubo S, 1996. Glucose and xylitol tolerance test for ketotic and healthy cows. J Dairy Sci 79:372-7.

Sano H, Nakai M, Kondo T, *et al*., 1991. Insulin responsiveness to glucose and tissue responsiveness to insulin in lactating, pregnant, and nonpregnant, nonlactating beef cows. J Anim Sci 69:1122-7.

Tse EO, Gregoire FM, Reusens B, *et al*., 1998. Changes of islet size and islet distribution resulting from protein-malnutrition in lean (Fa/Fa) and obese (fa/fa) Zucker rats. Obesity Res 5:563-71.

Xu C, Shu S, Xia C, *et al*., 2014. Investigation on the relationship of insulin resistance and ketosis in dairy cows. J Vet Sci Technol 5:162.

Youssef M and El-Ashker M, 2017. Significance of insulin resistance and oxidative stress in dairy cattle with subclinical ketosis during the transition period. Trop Anim Health Prod 49:239-44.

Zammit VA, 1996. Role of insulin in hepatic fatty acid partitioning: emerging concepts. Biochem J 314:1-14.

Zarrin M, Grossen-Rösti L, Bruckmaier RM, *et al*., 2017. Elevation of blood β hydroxybutyrate concentration affects glucose metabolism in dairy cows before and after parturition. J Dairy Sci 100:2323-33.