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The effects of post-partum drops in body condition on indices of energy metabolism in mid-lactation Holstein cows

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Abstract

Background: Evaluation of energy metabolism indices in mid-lactation Holstein cows is critical to monitor health status. **Aims:** The objective of this study was to assess the effects of low (≤0.75) *vs*. high (>0.75) drops in body condition score (BCS) until day 60 post-partum on energy metabolism indices during mid-lactation in Holstein cows. **Methods:** Twenty-eight Holstein cows were included in the study from the day of calving to day 120 of lactation. Whole blood samples were taken on 60, 90, and 120 days in milk (DIM). Serum was analyzed for insulin, glucose, non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) levels. **Results:** In cows with low BCS drop (LoD group), insulin did not change significantly through days 60 to 120 of lactation, but increased in high drop cows (HiD group) (P<0.001). Glucose concentrations decreased linearly in the LoD cows (P=0.039) and showed a quadratic increase in the HiD group on day 90 (P=0.028). Concentrations of non-esterified fatty acids showed both linear (P=0.04) and quadratic (P=0.002) changes in the HiD group. The HiD cows had significantly higher concentrations of insulin on day 120 (P=0.017) compared to the LoD group. Glucose concentration was lower (P<0.01) in HiD cows on 60 DIM. The concentration of non-esterified fatty acids was higher in HiD cows on day 90 (P<0.01). Surrogate indices of insulin resistance (calculated based on the concentrations of the measured metabolites) were different between the groups on day 90, indicating decreased insulin sensitivity in the HiD cows. **Conclusion:** Greater depletion of body reserves during early lactation may result in some inconsistencies in energy metabolism during mid-lactation periods. Controlling BCS loss during early lactation may help alleviate such alterations possibly through modifying insulin sensitivity of the tissues.

Key words: BCS, Energy metabolism indices, Holstein cows, Insulin sensitivity, Lactation

Introduction

Dairy cows lose their body condition for about 50 to 100 days in milk (DIM) due to increased lipolysis in the adipose tissue, resulting in elevated levels of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) in blood (Roche *et al*., 2009). Lipolysis, in turn, is stimulated by reduced insulin levels in blood, and a reduced sensitivity of peripheral tissues to insulin that suppresses glucose uptake in peripheral tissues (Roche *et al*., 2009; De Koster and Opsomer, 2013). Low insulin response of the peripheral tissues conducts glucose mostly to the mammary glands for milk production, and is characterized by low peripheral glucose concentrations (Balogh *et al*., 2008; De Koster and Opsomer, 2013). The secretion of insulin by the pancreas and insulin sensitivity of the peripheral tissues may regulate glucose metabolism. Insulin resistance may show as decreased insulin sensitivity or decreased insulin responsiveness (De Koster and Opsomer, 2013). Non-esterified fatty acids, BHB, insulin and glucose levels are associated with changes of the body condition score (BCS) post-calving and affect the general health of the cows (Roche *et al*., 2009; Abdelli *et al*., 2017). These conditions are anticipated to be resolved, with appropriate management, at about 4 weeks post-calving (Roche *et al*., 2009). However, high levels of NEFA and decreasing concentrations of glucose during 60 to 120 DIM (with some adverse effects on reproductive indices), and low levels of blood insulin up to 210 DIM have been reported by Mohebbi-Fani *et al*. (2018) and Oliveira *et al*. (2016), respectively. Body condition score losses >0.75 during the first and second months after calving have been associated with some adverse effects on reproductive indices (Opsomer *et al*., 2000; Kafi and Mirzaei, 2010). The present study assessed the effects of low (≤0.75) and high (>0.75) drops in BCS during early lactation on insulin, glucose, NEFA and BHB blood levels and some insulin resistance indices in mid-lactation Holstein cows under field conditions.

Materials and Methods

Animals and study protocol

The study was performed under the approval of the state committee on animal ethics, Shiraz University, Shiraz, Iran (IACUC No.: 4687/64). Also, the recommendations of the European Council Directive (86/609/EC) of November 24, 1986, regarding the

protection of animals used for experimental purposes were considered. The study was conducted in a dairy farm with controlled management and nutritional practices, owning 200 milking cows, located 60 km north of Shiraz, Fars province, Iran. Twenty-eight Holstein cows were included in the study from the day of calving (day 0) to day 120 of lactation. The animals were fed *ad libitum* with a balanced total mixed ration with forage to concentrate ratio of 43:57 on dry matter basis. The cows had an average 305-day milk production of 10,000 kg (based on the records of total lactation) and were clinically healthy at the sampling times. The BCS of each cow was detected by a skilled veterinarian based on the methodology described by Edmonson *et al*. (1989) at calving (day 0) and thereafter with 30-day intervals up to day 120. The initial BCS of the cows at calving day was 3.47 ± 0.04. The animals were sampled for whole blood on 60, 90 and 120 (±5) DIM in the morning between 09:00 and 11:00 by coccygeal vessels' venipuncture. Serum were separated after centrifugation at 3000 rmp for 10 min at room temperature and stored at -20°C until analysis. The few samples with obvious hemolysis or clot formation were discarded.

Biochemical analysis

Plasma insulin and NEFA concentrations were measured using a solid phase sandwich ELISA method (bovine ELISA kits; Bioassay Technology Laboratory, Shanghai Crystal Day Biotech Company, China). Beta-hydroxybutyrate was measured using the Williamson-Mellanby enzymatic method (Commercial kit, Biorex Fars Company, Shiraz, Iran). Glucose was assayed by glucose oxidase method (Commercial kit, Pars Azmoon Company, Tehran, Iran).

Insulin resistance indices

The surrogate indices of insulin resistance were calculated from the concentrations of plasma insulin, glucose, NEFA and BHB (De Koster and Opsomer, 2013) for various sampling days:

HOMA = Glucose (mmol/L) × insulin (μU/ml)

QUICKI = 1/[log (glucose mg/dl) + log (insulin μU/ml)]

RQUCKI = 1/[log (glucose mg/dl) + log (insulin μU/ml) + log (NEFA mmol/L)]

RQUICKI-BHB = 1/[log (glucose mg/dl) + log (insulin μU/ml) + log (NEFA mmol/L) + log (BHB mmol/L)]

Where,
HOMA: The homeostasis model assessment
QUICKI: The quantitative insulin sensitivity check index
RQUICKI: The revised quantitative insulin sensitivity check index
RQUICKI-BHB: The revised quantitative insulin sensitivity check index including BHB

Statistical methods

The cows were divided into two groups based on the level of drop in BCS on 60 DIM compared to the calving day; low drops (≤0.75; n=11; LoD group) and high drops (>0.75; n=17; HiD group). The time trends of changes in the studied variables were compared between and within the BCS groups as well as all studied cows through 60 to 120 DIM using analysis of variance (ANOVA) for repeated measures. The differences between the sampling days were assessed using a one-way ANOVA and Duncan's multiple range tests. The differences of the variables between the BCS groups at each sampling day were compared using independent t-tests. The correlations of the variables with BCS at days 60, 90, and 120 were assessed using Pearson's correlations. All analyses were performed and expressed as mean±SD using the SPSS statistical software (version 16). The probability values of $P<0.05$ were considered significant.

Results

The changes of BCS in the studied groups are shown in Fig. 1. In the LoD group, the least BCS after calving (2.80 ± 0.31) was detected on 30 DIM. Thereafter, it remained constant until 60 DIM and then increased non-significantly. In the HiD group, however, the least BCS (3.37 ± 0.22) was detected on 60 DIM with non-significant improvement until 120 DIM. The differences of BCS were significant between groups on days 30 ($P=0.008$), 60, 90 and 120 ($P\leq0.001$).

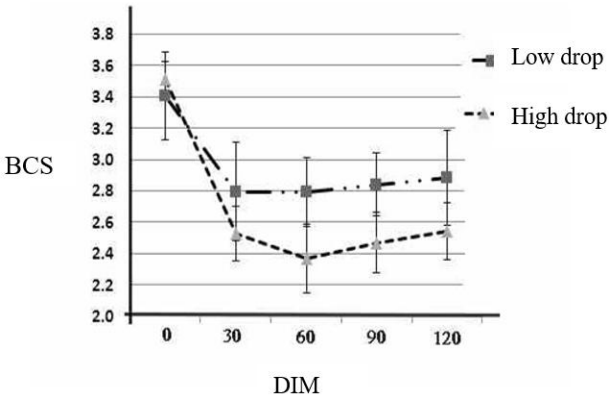

Fig. 1: Changes in body condition score (BCS) from calving to 120 days in milk (DIM) in low drop (≤0.75) and high drop (>0.75) BSC groups

For all studied cows, the variables (except BHB) changed significantly ($P<0.05$) through days 60 to 120 of lactation (Figs. 2A-D and 3A-D). The main effect of BCS loss at 60 DIM on the trends of changes of the variables was not different between groups ($P>0.05$). However, within each BCS group, noticeable findings were observed in the trends of changes (Table 1). Insulin concentrations were almost unchanged ($P=0.057$) in LoD cows (d60 BCS drop ≤0.75), but increased significantly ($P<0.001$) in the HiD group (d60 BCS drop >0.75) between days 60 to 120 of lactation. Glucose concentrations decreased linearly in LoD cows ($P=0.039$) and showed a quadratic increase in the HiD group on day 90 ($P=0.028$). Non-esterified fatty acids' concentrations tended to increase in LoD cows ($P=0.054$), but showed

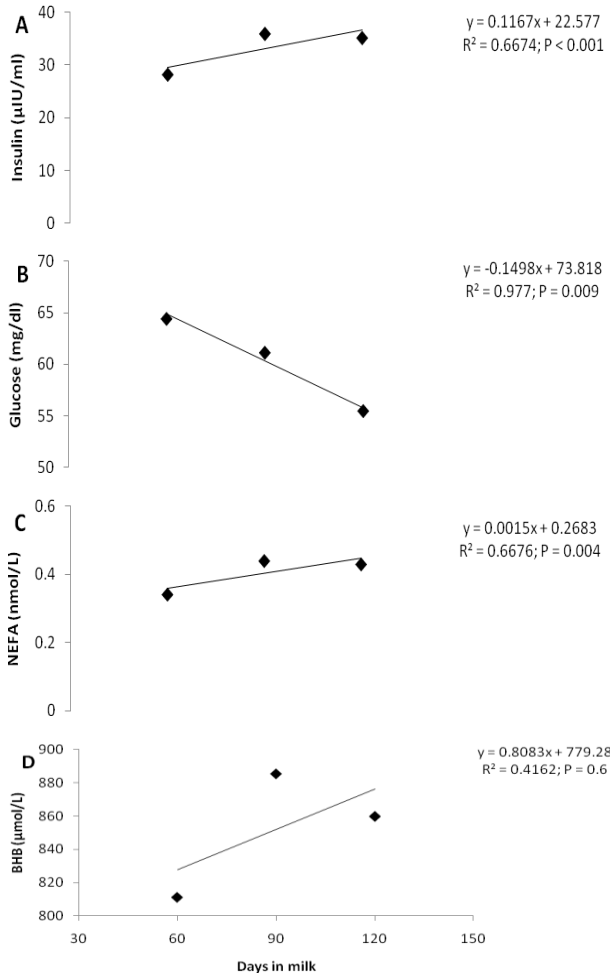

Fig. 2: Time trend of changes of insulin (**A**), glucose (**B**), NEFA (**C**), and BHB (**D**) during sampling time. BHB: Beta-hydroxybutyrate, and NEFA: Non-esterified fatty acids

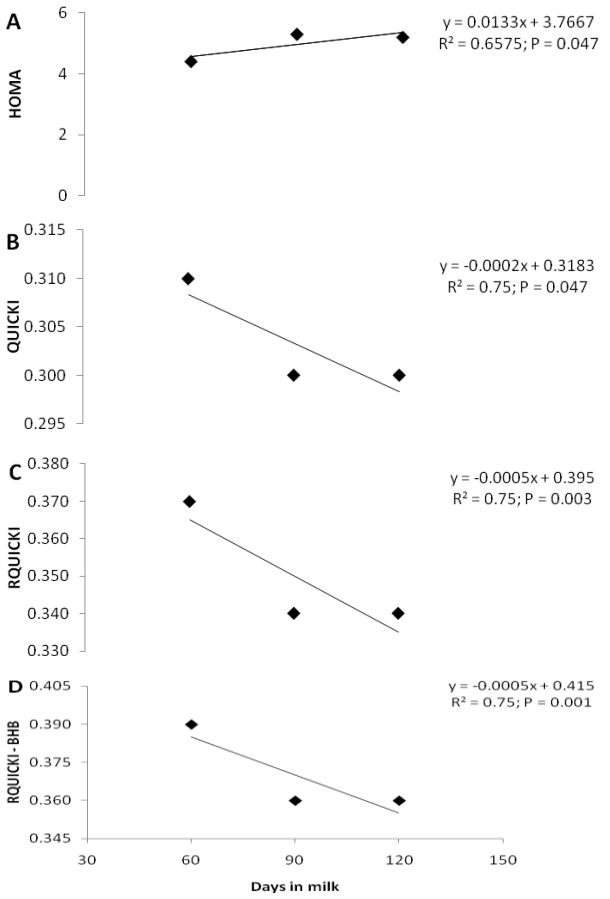

Fig. 3: Time trend of changes of HOMA (**A**), QUICKI (**B**), RQUICKI (**C**), and RQUICKI-BHB (**D**) during sampling time. RQUICKI: Revised quantitative insulin sensitivity check index, BHB: Beta-hydroxybutyrate, QUICKI: Quantitative insulin sensitivity check index, and HOMA: Homeostasis model assessment

Table 1: Concentrations (mean±SD) of insulin, glucose, NEFA, and BHB and calculated indices of insulin sensitivity during days 60 to 120 of lactation in Holstein cows with BCS loss ≤0.75 and >0.75 at day 60

Variable	BCS loss at day 60	Days in milk (DIM)			P-value (for trend of changes through the study)
		60	90	120	
Insulin (µIU/ml)	≤0.75 (n=11)	25.85 ± 10.35	31.27 ± 10.57	32.98 ± 7.89*	0.057
	>0.75 (n=17)	29.65 ± 10.37^{a}	38.95 ± 9.84^{b}	43.14 ± 11.51^{b*}	<0.001
Glucose (mg/dl)	≤0.75 (n=11)	73.53 ± 15.45^{a**}	55.41 ± 18.66^{b}	61.45 ± 16.53^{b}	0.039
	>0.75 (n=17)	58.56 ± 11.60^{ab**}	64.84 ± 13.84^{a}	51.56 ± 14.90^{b}	0.112 (Q, 0.028)
NEFA (nmol/L)	≤0.75 (n=11)	0.35 ± 0.14	0.36 ± 0.12**	0.46 ± 0.15	0.054 (Q, 0.073)
	>0.75 (n=17)	0.34 ± 0.11^{a}	0.49 ± 0.10^{b**}	0.41 ± 0.11^{ab}	0.040 (Q, 0.002)
BHB (µmol/L)	≤0.75 (n=11)	792.20 ± 256.46	753.23 ± 415.73	857.14 ± 611.12	0.689
	>0.75 (n=17)	823.53 ± 620.20	970.59 ± 545.77	861.34 ± 395.66	0.832
HOMA	≤0.75 (n=11)	4.68 ± 2.03	3.98 ± 1.28**	5.02 ± 1.93	0.607
	>0.75 (n=17)	4.23 ± 1.62^{a}	6.18 ± 1.99^{b**}	5.36 ± 1.83^{ab}	0.037 (Q, 0.026)
QUICKI	≤0.75 (n=11)	0.310 ± 0.021	0.314 ± 0.013**	0.306 ± 0.016	0.496
	>0.75 (n=17)	0.313 ± 0.016^{a}	0.297 ± 0.012^{b**}	0.303 ± 0.015^{b}	0.048 (Q, 0.008)
RQUICKI	≤0.75 (n=11)	0.368 ± 0.043	0.369 ± 0.021**	0.344 ± 0.026	0.093
	>0.75 (n=17)	0.371 ± 0.036^{a}	0.328 ± 0.018^{b**}	0.346 ± 0.025^{b}	0.017 (Q, <0.001)
RQUICKI-BHB	≤0.75 (n=11)	0.387 ± 0.044	0.397 ± 0.036**	0.364 ± 0.042	0.145
	>0.75 (n=17)	0.399 ± 0.050^{a}	0.336 ± 0.025^{b**}	0.360 ± 0.034^{b}	0.005 (Q, <0.001)

$^{a, b}$ Different letters in the rows indicate to significant differences between sampling days (P<0.05). * and ** Respectively P<0.05 and P<0.01 at the corresponding sampling day. NEFA: Non-esterified fatty acids, BHB: Beta-hydroxybutyrate, HOMA: Homeostasis model assessment, QUICKI: Quantitative insulin sensitivity check index, RQUICKI: Revised quantitative insulin sensitivity check index, BCS: Body condition score, and Q: Quadratic trend of change

Table 2: Correlations (r values) between BCS and BCS drop of different steps during lactation and insulin sensitivity indices calculated for day 90 of lactation

Variable	HOMA day 90	QUICKI day 90	RQUICKI day 90	RQUICKI-BHB day 90
BCS at calving	NS	NS	NS	-0.499**
BCS day 30	NS	NS	NS	NS
BCS day 60	-0.679**	0.659**	0.634**	0.406*
BCS day 90	-0.699**	0.699**	0.631**	0.424*
BCS day 120	-0.542**	0.551**	0.549**	NS
BCS loss day 30	NS	NS	-0.490**	-0.562**
BCS loss day 60	0.676**	-0.672**	-0.819**	-0.697**
BCS loss day 90	0.654**	-0.669**	-0.789*	-0.705**
BCS loss day 120	0.494**	-0.515**	-0.676**	-0.585**

BCS: Body condition score, HOMA: Homeostasis model assessment, QUICKI: Quantitative insulin sensitivity check index, RQUICKI: Revised quantitative insulin sensitivity check index, BHB: Beta-hydroxybutyrate, and NS: Non-significant. * Significant correlation at P≤0.05, and ** Significant correlation at P≤0.01

both linear (P=0.04) and quadratic (P=0.002) changes in the HiD group. Beta-hydroxybutyrate changes were not significant within the BCS groups. Neither of the surrogate indices of insulin sensitivity showed significant changes in the LoD group but all showed both linear and quadratic changes in HiD cows.

Comparing the various sampling days (Table 1), the HiD cows tended (P=0.061; not shown) to have higher levels of insulin on day 90 of lactation, but had significantly higher concentrations of insulin on day 120 (P=0.017) compared to the LoD group. Glucose concentration was lower (P<0.01) in HiD cows on 60 DIM compared to the LoD group. Concentrations of non-esterified fatty acids were significantly higher in HiD cows on day 90 (P<0.01). Beta-hydroxybutyrate changes were not significantly different between and within the BCS groups. Homeostasis model assessment was higher (P<0.01) and QUICKI, RQUICKI, and RQUICKI-BHB were lower (P<0.01) on 90 DIM in HiD cows compared to the LoD group (Table 1). The insulin sensitivity indices of day 90 showed some negative and positive correlations with BCS and BCS drop of various days of the study (Table 2). Such correlations were not seen on days 60 and 120.

Discussion

While the LoD cows (d60 BCS drop ≤0.75) had less changes in blood metabolites, the HiD cows (d60 BCS drop >0.75) showed some changes with potential effects on normal body functions comparable to those that may happen in peripartum cows (Van Knegsel *et al*., 2014). These included less blood glucose levels on day 60, higher NEFA concentrations of over 0.4 mmol/L, the alarm level for affected energy metabolism in pre-fresh cows (Oetzel, 2004) on day 90, and increasing insulin concentrations throughout the study.

Comparable to those that may be detected in pre-fresh cows, high levels of NEFA have been reported in mid-lactation cows under field conditions, probably as a consequence of undetectable under-feeding (Mohebbi-Fani *et al*., 2018). However, it is concluded in the present study that elevated NEFA levels in HiD cows during mid-lactation could be a result of the reduced insulin sensitivity of tissues. Reduced sensitivity to insulin necessitates higher insulin levels in the blood for performing normal body functions (Baruselli *et al*., 2016) and may last one to 4 weeks post-calving (Roche *et al*., 2009), nevertheless, its onset may be traced to the end of pregnancy (De Koster and Opsomer, 2013). Oliviera *et al*. (2016) reported increased levels of insulin in cows on 150 DIM and concluded that insulin insensitivity will occur with the increase of DIM and BCS. In the present study, however, elevated insulin concentrations were detected only in the HiD cows from about 90 DIM, and the LoD group in BCS appeared not to be affected. Increased HOMA and decreased QUICKI, RQUICKI, and RQUICKI-BHB in HiD cows around day 90 indicated insulin resistance. It is concluded that, with the progress in DIM, thin cows may develop insulin insensitivity, leading to less uptake of glucose by tissues, less lipogenesis and more NEFA levels in the blood. Elevated blood levels of NEFA in HiD cows on day 90 concurrent with the increase in glucose levels (P=0.028) and a non-significant increase in BHB concentrations on the same day could support this conclusion (Table 1). Rising plasma NEFA levels contribute to insulin insensitivity by suppression of glucose uptake in adipose tissues and muscles (Guyot *et al*., 2017). The elevated NEFA level seen in LoD cows on day 120 was not coincided by increased insulin levels.

The differences of surrogate indices of insulin sensitivity between the groups on day 90 and their strong correlations with post-partum BCS and BCS drop on the same day could mean that the HiD cows were less sensitive to insulin during mid-lactation. Revised quantitative insulin sensitivity check index, which showed the strongest correlations with BCS loss, varies between 0.35 and 0.68 (Holtenius and Holtenius, 2007; Balogh *et al*., 2008; Gross *et al*., 2011; Cincović *et al*., 2017), and has been suggested as the most appropriate predictor of metabolic status in dairy cows (Holtenius and Holtenius, 2007; Cincović *et al*., 2017). These indices, adopted from human medicine as inexpensive and non-invasive methods (De Koster and Opsomer, 2013), are not affected by the homeorhetic adaptations of individual substances (Holtenius and Holtenius, 2007; De Koster and Opsomer, 2013; Marett *et al*., 2015) and have been addressed in studies with dairy cows

(Holtenius and Holtenius, 2007; De Koster and Opsomer, 2013; Kinoshita *et al*., 2018). Based on new findings in dairy cows (Alves-Nores *et al*., 2017) and human medicine (Rudvik and Månsson, 2018), complementary research using tests such as the intravenous glucose tolerance test (IVGTT) and hyperinsulinemic euglycemic clamp (HEC) across various BCS levels in mid-lactation cows are suggested to study insulin insensitivity.

Overweighing at calving may lead to the inhibition of insulin effects for longer times concurring with greater circulating levels of insulin and NEFA (Rico *et al*., 2015). A negative association has been reported between the level of fat accumulation in late pregnant dairy cows (three weeks before calving) and the insulin response of glucose metabolism (De Koster *et al*., 2015). Holtenius and Holtenius (2007) suggested disturbed insulin function in obese cows with lower RQUICKI in early lactation. In the present study, the cows were not over-conditioned at calving (BCS was 3.41 ± 0.28 in LoD cows, 3.51 ± 0.11 in HiD cows and 3.47 ± 0.04 in all cows), but those with greater BCS drops up to 60 DIM had indications of reduced insulin sensitivity on d90. Visceral fat, having the same effects of fatness but not reflected on BCS (Van Saun and Sniffen, 2014), may also have a role in such conditions.

It is concluded that greater depletion of body reserves during early lactation may result in some inconsistencies in energy metabolism during mid-lactation periods. This may happen even in well-conditioned cows that have desired BCS at calving. Controlling BCS loss during early lactation may help alleviate such alterations possibly through modifying insulin sensitivity of the tissues. Assessing the effects of such conditions on general health and cow performance is suggested.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Abdelli, A; Raboisson, D; Kaidi, R; Ibrahim, B; Kalem, A and Iguer-Ouada, M (2017). Elevated non-esterified fatty acid and β-hydroxybutyrate in transition dairy cows and their association with reproductive performance and disorders: a meta-analysis. Theriogenology. 93: 99-104.

Alves-Nores, V; Castillo, C; Hernandez, J and Abuelo, A (2017). Comparison of surrogate indices for insulin sensitivity with parameters of the intravenous glucose tolerance test in early lactation dairy cattle. Dom. Anim. Endocrinol., 61: 48-53.

Balogh, O; Szepes, O; Kovacs, K; Kulcsar, M; Reiczigel, J; Alcazar, JA and Fesus, L (2008). Interrelationships of growth hormone AluI polymorphism, insulin resistance, milk production and reproductive performance in Holstein-Friesian cows. Vet. Med-Czech., 53: 604-616.

Baruselli, PS; Vieira, LM; SáFilho, MF; Mingoti, RD; Ferreira, RM; Chiaratti, MR and Sartori, R (2016). Associations of insulin resistance later in lactation on fertility of dairy cows. Theriogenology. 86: 263-269.

Cincović, M; Kirovski, D; Vujanac, I; Belić, B and Djoković, R (2017). Relationship between the indexes of insulin resistance and metabolic status in dairy cows during early lactation. Acta Vet. Brno. 67: 57-70.

De Koster, JD; Hostens, M; Van Eetvelde, M; Hermans, K; Moerman, S; Bogaert, H; Depreester, E; Van den Broeck, W and Opsomer, G (2015). Insulin response of the glucose and fatty acid metabolism in dry dairy cows across a range of body condition scores. J. Dairy Sci., 98: 4580-4592.

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

Edmonson, AJ; Lean, IJ; Weaver, LD; Farver, T and Webster, G (1989). A body condition scoring chart for Holstein dairy cows. J. Dairy Sci., 72: 68-78.

Gross, J; van Dorland, HA; Schwarz, FJ and Bruckmaier, RM (2011). Endocrine changes and liver mRNA abundance of somatotropic axis and insulin system constituents during negative energy balance at different stages of lactation in dairy cows. J. Dairy Sci., 94: 3484-3494.

Guyot, H; Detilleux, J; Lebreton, P; Garnier, C; Bonvoisin, M; Rollin, F and Sandersen, C (2017). Comparison of various indices of energy metabolism in recumbent and healthy dairy cows. PloS One. 12: 1-11.

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

Kafi, M and Mirzaei, A (2010). Effects of first postpartum progesterone rise, metabolites, milk yield, and body condition score on the subsequent ovarian activity and fertility in lactating Holstein dairy cows. Trop. Anim. Health Prod., 42: 761-767.

Kinoshita, A; Keese, C; Meyer, U; Starke, A; Wrenzycki, C; Dänicke, S and Rehage, J (2018). Chronic effects of fusarium mycotoxins in rations with or without increased concentrate proportion on the insulin sensitivity in lactating dairy cows. Toxins (Basel). 10: 1-23.

Marett, LC; Auldist, MJ; Moate, PJ; Wales, WJ; Macmillan, KL; Dunshea, FR and Leury, BJ (2015). Response of plasma glucose, insulin, and nonesterified fatty acids to intravenous glucose tolerance tests in dairy cows during a 670-day lactation. J. Dairy Sci., 98: 179-189.

Mohebbi-Fani, M; Omidi, A; Mirzaei, A; Nazifi, S and Nowroozi, K (2018). A field study on glucose, non-esterified fatty acids, beta-hydroxybutyrate and thyroid hormones in dairy cows during the breeding period in Fars province, Iran. Iran. J. Vet. Res., 20: 55-59.

Oetzel, GR (2004). Monitoring and testing dairy herds for metabolic disease. Vet. Clin. Food Anim. Pract., 20: 651-674.

Oliviera, LH; Nascimento, AB; Monteiro, PLJ; Guardieiro, MM; Wiltbank, MC and Sartori, R (2016). Development of insulin resistance in dairy cows by 150 days of lactation does not alter oocyte quality in smaller follicles. J. Dairy Sci., 99: 9174-9183.

Opsomer, G; Grohn, YT; Hertl, J; Coryn, M; Deluyker, H and de Kruif, A (2000). Risk factors for post partum

ovarian dysfunction in high producing dairy cows in Belgium: a field study. Theriogenology. 53: 841-857.

Rico, JE; Bandaru, VVR; Dorskind, JM; Haughey, NJ and McFadden, JW (2015). Plasma ceramides are elevated in overweight Holstein dairy cows experiencing greater lipolysis and insulin resistance during the transition from late pregnancy to early lactation. J. Dairy Sci., 98: 7757-7770.

Roche, JR; Friggens, NC; Kay, JK; Fisher, MW; Stafford, KJ and Berry, DP (2009). Body condition score and its association with dairy cow productivity, health, and welfare. J. Dairy Sci., 92: 5769-5801.

Rudvik, A and Månsson, M (2018). Evaluation of surrogate measures of insulin sensitivity-correlation with gold standard is not enough. BMC Med. Res. Methodol., 18: 64. https://doi.org/10.1186/s12874-018-0521-y.

Van Knegsel, AT; Hammon, HM; Bernabucci, U; Bertoni, G; Bruckmaier, RM; Goselink, RM and Trevisi, E (2014). Metabolic adaptation during early lactation: key to cow health, longevity and a sustainable dairy production chain. CAB Rev., 9: 1-15.

Van Saun, RJ and Sniffen, CJ (2014). Transition cow nutrition and feeding management for disease prevention. Vet. Clin. Food Anim. Pract., 30: 689-719.