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REVIEWS

Significance of insulin resistance and oxidative stress in dairy cattle with subclinical ketosis during the transition period

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Abstract Health problems occurring during the transition period in dairy cattle are of utmost importance as they can decrease the animal's reproductive performance and favor the development of various metabolic diseases with resultant significant reproductive disorders. Among the commonly reported metabolic diseases occurring during that time, hyperketonemia is the most prevalent and could provoke a significant economic impact. The failing of a dairy cow to transit optimally between pregnancy and lactation is economically very relevant and should be considered. Until now, the role of insulin resistance (IR) in the etiology of subclinical ketosis (SCK) in dairy cattle is not clearly understood. This review aims to shed some light on the role of IR and oxidative stress in dairy cows with SCK during the transition period. The data presented in this review demonstrates that dairy cows could be vulnerable to the development of negative energy balance during transition. Moreover, the transitional cows could succumb to both IR and oxidative stress; however, the exact role of IR in cows with SCK needs further investigations. It is imperative to elaborate a suitable nutritional strategy to facilitate an easy transit of cows through this critical period and to minimize health problems and improve productivity during lactation.

Keywords Subclinical ketosis · Dairy cattle · Insulin resistance · Oxidative stress

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The transition period in dairy cattle

The peri-parturient period, defined as 3 weeks around the time of parturition, is physiologically stressful and represents a critical time for dairy cattle (Elischer et al. 2015; Heiser et al. 2015). During the transition period, profound physiological alterations can drastically modify metabolism (Elischer et al. 2015; Heiser et al. 2015). The productivity of dairy cows often depends on level of milk production, postpartum recovery, reproductive efficiency, and the absence of pathology (Bezerra et al. 2014).

Metabolic diseases are a substantial challenge in the dairy industry in which modern dairy cattle are managed and fed for optimal milk production (Herdt 2013). Undoubtedly, this challenge is related to the progressive improvement in dairy cow genetics and the ever-increasing average milk yields of modern dairy cattle. During the transition period, there is a critical gap between the nutrient requirements and supply due to the variations in nutrient content of feeds as well as dry matter intake (DMI) (Sundrum 2015). Dairy cows need to adjust their metabolism to cope with the dramatic increase in energy and nutrient requirements that are required for optimal milk production (Sundrum 2015). DMI decreases in the first few weeks of the dry period from 2% of body weight to 1.4% in the seventh to tenth day before calving (Block 2010).

Subclinical ketosis in transition dairy cattle

Recent reports have examined the metabolic alterations and health problems linked to increased animal productivity (Bezerra et al. 2014; Elischer et al. 2015; Sundrum 2015). Of the metabolic diseases occurring in transition, subclinical ketosis (SCK) is the most prevalent and has a significant economic impact in dairy cattle. The disease has been defined as abnormal levels of circulating ketone bodies in the absence of clinical signs (Andersson 1988). It is caused by the prolonged negative energy balance (NEB) in early lactation (Herdt 2000). It has been reported that NEB is mainly caused by a rapid increase in nutrient demands that exceed food intake in order to support fetal growth and milk production (Herdt 2000; Sundrum 2015).

After calving, the nutrient energy deficit is often met by mobilizing body fat reserves and decreasing the non-essential use of glucose in non-mammary tissue (Zachut et al. 2013). In the normal state, fat deposited in adipose tissue (in the form of triglycerides) is mobilized as non-esterified fatty acid (NEFA) plus glycerol. The NEFA is used by the peripheral tissue as a source of energy or to be re-esterified by the liver (Block 2010). Under optimal conditions, NEFA is completely oxidized to provide energy for the liver and is partially oxidized to liberate ketone bodies that are released into the blood circulation to serve as fuels for other tissues or reconverted to storage fat triglycerides (Ingvartsen 2006). Ruminants have an inherently low capacity to synthesize and secrete very-low-density lipoprotein (VLDL) in order to export triglyceride from the liver and a similar capacity to reconvert NEFA back to triglyceride (Drackley 2005). Triglycerides can travel to other tissues with the help of VLDL or can be stored in the liver. Beyond the stimulation of gluconeogenesis, NEFA and triglyceride, as well as ketones, can act as alternative fuel sources for many body tissues in order to spare glucose (Herdt 2000).

In NEB, which occurs in early post-parturition, the demand for glucose often exceeds the gluconeogenic ability of the liver (Holtenius and Holtenius 1996). The gluconeogenic pathways in the liver are maximally stimulated but blood glucose is still low, owing to a lack of sufficient glucose sources. Therefore, the low circulating levels of blood glucose and insulin can lead to a high level of fat catabolism and promote ketone body formation (Herdt 2000). A consequence of this theory would be fat accumulation in the liver without maximal stimulation of glucose production (Holtenius and Holtenius 1996). As NEB increases, more NEFA are released from body fat with a resultant increase in serum levels of NEFA (Bezerra et al. 2014). As the degree of fatty liver increases, normal functions are adversely affected while fat infiltration overwhelms the ability of the liver to detoxify ammonia to urea (Drackley 2005).

Insulin resistance in dairy cattle during the transition period

Insulin resistance (IR) has been defined as a state where a normal level of insulin is associated with a decreased response in insulin-sensitive tissues (Herdt 2013). Insulin has been reported to play a fundamental role in the physiological adaptation of dairy cows especially around the time of parturition (Zachut et al. 2013). After calving, cows undergo a transient period of IR to prioritize an insulin-independent uptake of glucose by the mammary gland in order to improve milk production (De Koster and Opsomer 2013). The period of IR in transition dairy cattle has elements in common with human type 1 and type 2 diabetes, but it varies in the fact that cows have a lower level of blood glucose (Abuelo et al. 2016).

Dairy cows are susceptible to alterations in insulin action in peripheral tissues especially during the transition period (Zachut et al. 2013; Youssef et al. 2016). Previous studies have proposed a relationship between body condition score (BCS) and glucose tolerance and have found that cows with moderate BCS at calving showed a greater impairment in insulin function than very thin or very fat cows (Jaakson et al. 2013). The intense lipid mobilization and excessive accumulation of NEFA could also result in increased expression of proinflammatory cytokines, which exacerbates the production of acute phase proteins and inflammatory response (Abuelo et al. 2016).

The euglycemic hyperinsulinemic clamp is considered a “golden standard” method to measure IR by estimating serum glucose and blood insulin levels in animals (Xu et al. 2014), as the endogenous insulin production could be inhibited by an exogenous insulin infusion, using the principle of the glucose-insulin feedback mechanism (Sternbauer and Luthman 2002). However, both the intravenous glucose tolerance test and the euglycemic hyperinsulinemic clamp are time-consuming and invasive procedures; therefore, they are not suitable for either field use or for large-scale epidemiological investigations (Xu et al. 2014).

There is currently a trend to use several surrogate indices to estimate insulin sensitivity in dairy cattle during the transition period (Xu et al. 2014; Abuelo et al. 2014, 2016; Youssef et al. 2016). These indices have been extrapolated from human research and could provide similar but not identical results. In brief, the homeostasis model assessment (HOMA) was developed to assess insulin resistance; it depends on blood insulin and glucose levels as follows: (Insulin μU/ml) × (glucose mmol/l); Log HOMA: Log transformation of HOMA = Log (Insulin μU/ml) × (glucose mmol/l); $HOMA^{-1}$: reciprocal score of HOMA was also developed as 1/(Insulin μU/ml) × (glucose mmol/l).

Taking into account the serum values of glucose, NEFA and insulin from prepartum to postpartum periods, a revised quantitative insulin sensitivity check index (RQUICKI) has recently been developed (Xu et al. 2014). The quantitative insulin sensitivity check index (QUICKI) was evaluated using blood glucose and insulin levels (Abuelo et al. 2016; Youssef et al. 2016). Another surrogate index, namely revised quantitative insulin sensitivity check index with BHBA ($RQUICKI_{BHB}$), has also been evaluated (Balogh et al. 2008; Youssef et al. 2016). Values of the RQUIKI index have been reported for ketotic dairy cattle during the transitional period (Xu et al.

Table 1 Levels of cited metabolic indices in dairy cows during the transition period

BHB (mmol/l)	NEFA (mmol/l)	Glucose (mg/dl)	Insulin (μU/ml)	References
1.45 ± 1.5	–	60.0 ± 13	–	González et al. (2011)[a, *EL*]
0.82 ± 0.3	0.30 ± 0.2	60.1 ± 8.6	–	González et al. (2011)[b, *EL*]
1.3 ± 0.13	–	–	–	Sentürk et al. (2010)[a, *EL*]
1.59 ± 0.2	0.75 ± 0.1	57.6 ± 2.1	–	Nogalski et al. (2012)[a, *EL*]
–	–	67.0	–	Zachut et al. (2013)[b, *LG*]
–	–	55.9	–	Zachut et al. (2013)[b, *EL*]
2.7 ± 1.3	0.99 ± 0.3	50.3 ± 16	13.87 ± 1.7	Xu et al. (2014)[a, *EL*]
0.92 ± 0.3	0.34 ± 0.1	88.7 ± 19.3	29.07 ± 0.5	Chalmeh et al. (2015)[b, *EL*]
1.8 ± 0.3	0.50 ± 0.1	53.16 ± 15	53.72 ± 18	Youssef et al. (2016)[a, *LG*]

LG investigation during the late gestation time, *EL* investigation during the early lactation period

[a] Subclinical ketotic cows during transition phase

[b] Healthy transition cows

2014; Youssef et al. 2016); while values of QUICKI, RQUICKI, and $RQUICKI_{BHB}$ have been calculated in transitional dairy cows at 2 weeks prepartum (Abuelo et al. 2016). It has also been reported that clinically ketotic dairy cows can exhibit a state of NEB, liver dysfunction, oxidative stress, and decreased insulin sensitivity, thereby implying a potential relevance of IR in ketotic dairy cows (Xu et al. 2014).

The relation between oxidative stress and insulin resistance in cattle with subclinical ketosis

A significant mobilization of NEFA in ketotic dairy cows has also been associated with enhanced production of free oxygen radicals, such as reactive oxygen species (ROS) which could initiate a state of oxidative stress and lead to inhibition of glucose intake through interfering with glucose transporter function, and inhibits insulin signal transduction pathway in the liver and peripheral tissues (Xu et al. 2014). The initiation of oxidative stress pathway will prompt a state of IR, along with insulin secretion injury and vasculopathy. In addition, the development of oxidative stress is thought to play a key role in the dysfunctional inflammatory responses that occur around the time of calving (Sordillo and Raphael 2013; Abuelo et al. 2016).

The diagnosis of subclinical ketosis in dairy cattle

The detection of SCK is carried out by checking the levels of ketone bodies in the blood, urine, and milk. Because of its economic and clinical consequences, it is imperative to diagnose SCK as early as possible, especially during the transition period.

Laboratory diagnosis

Measurements of beta-hydroxybutyric acid

Measurement of serum BHBA remains the gold standard for the diagnosis of SCK in dairy cattle (Gordon 2013). It is more

Table 2 Levels of cited insulin sensitivity indices in dairy cows in transition

RQUICKI	QUICKI	$RQUICKI_{BHB}$	HOMA	Log HOMA	$HOMA^{-1}$	References
0.48 ± 0.15	–	–	–	–	–	Holtenius and Holtenius (1996)[*EL*]
0.36 ± 0.02	–	–	–	–	–	Xu et al. (2014)[a, *EL*]
0.40	0.34	0.44	53.5	1.6	0.022	Abuelo et al. (2016)[b, *LG*]
0.42	0.36	0.48	36.7	1.5	0.037	Abuelo et al. (2016)[b, *EL*]
0.32 ± 0.02	0.29 ± 0.02	0.55 ± 0.3	161 ± 79	2.13 ± 0.3	0.0083 ± 0.0	Youssef et al. (2016)[a, *LG*]

LG investigation during the late gestation time, *EL* investigation during the early lactation period

[a] Subclinical ketotic cows during transition phase

[b] Healthy transition cows

Table 3 Levels of cited lipid profile as well as oxidative stress markers in dairy cows during the transition period

Triglyceride (mg/dl)	Cholesterol (mg/dl)	VLDL (mg/dl)	MDA (nmol/l)	SOD (U/ml)	References
13.3	126.7	1.2	–	–	Sevinç et al. (1997)[a, *EL*]
–	82.8	–	–	–	Filipejová and Kováčik (2009)[b, *EL*]
–	–	–	2.89 ± 0.48	75.61 ± 6.34	Xu et al. (2014)[a, *EL*]
123.4 ± 21.9	153.3 ± 32.02	24.6 ± 4.38	–	–	Chalmeh et al.(2015)[b, *EL*]

LG investigation during the late gestation time, *EL* investigation during the early lactation period

[a] Subclinical ketotic cows during transition phase

[b] Healthy transition cows

stable in blood than acetone or acetoacetate (Herdt 2000). The most commonly used cut-point for SCK in dairy cattle is ≥1400 μmol/L of serum BHBA (Carrier et al. 2004). Early lactating cows with blood BHBA above this cut-point are at threefold greater risk to develop abomasal displacement and clinical ketosis, while cows with serum BHBA >2000 μmol/L could be considered at risk for reduced milk yield (Duffield et al. 1997). Some reports have used a lower cut-point (1200 μmol/L) of serum BHBA to define SCK (McArt et al. 2012). However, the chosen cut-point usually has a minor effect on the interpretation of herd-based results. Clinical ketosis is generally associated with much higher levels of BHBA (i.e., 3000 μmol/L or more).

Measurements of ketone bodies in urine and milk

The total ketone body concentrations are reported to be approximately four times higher in urine compared to those in the blood. Tests using milk ketone are often preferred for routine use on the farm due to the ease of milk collection and minimal training required to perform the test (Gordon 2013).

Cowside tests

Four cowside tests are acceptable for the routine use: Ketostix for detection of urine acetoacetate, Keto Test or Porta BHB for detection of milk BHBA, and precision Xtra for detection of blood BHBA. Although the high sensitivity (90%) and specificity (86%) of Ketostix test strip when read at 5 s with a cut-point of "trace," the development of highly accurate cowside milk and blood tests has limited the use of this test because of the challenge of collecting urine from all animals (Gordon 2013).

Metabolic indices in dairy cattle during the transitional period

Several blood metabolites including NEFA, glucose, triglyceride, and total cholesterol have been extensively estimated in dairy cattle to assess the energy balance status during the periparturient period (González et al. 2011; Xu et al. 2014; Djoković et al. 2015). To ensure the convenience for the readers, we summarized the most commonly relevant values of serum biochemical variables in dairy cattle during the transitional period (Tables 1, 2, 3, 4, and 5). It has been stated that almost all available circulating glucose is taken up by the mammary gland (Drackley et al. 2001); with a resultant low level of blood glucose that is close to the lower limit of the reference values. Such a decrease in blood glucose levels would result in lower circulating insulin levels, thus redirecting blood glucose towards the mammary gland with a subsequent increase in fat mobilization through lipolysis (Sordillo and Raphael 2013). It has also been reported that blood glucose,

Table 4 Levels of cited blood metabolites in dairy cows during the transition phase

Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Blood urea (mg/dl)	Creatinine (mg/dl)	Reference
7.2	3.3	–	46.4	–	Sevinç et al. (1997)[a, *EL*]
–	–	–	63	–	Filipejová and Kováčik (2009)[b, *EL*]
7.3 ± 0.3	3.2 ± 0.2	–	–	–	Xu et al. (2014)[a, *EL*]
6.8 ± 0.8	3.7 ± 0.4	3.1 ± 0.9	–	–	González et al. (2011)[a, *EL*]
8.7 ± 0.2	2.1 ± 0.2	6.6 ± 0.2	20.7 ± 1.1	–	Sentürk et al. (2010)[a, *EL*]

LG investigation during the late gestation time, *EL* investigation during the early lactation period

[a] Subclinical ketotic cows during transition phase

[b] Healthy transition cows

Table 5 Levels of cited liver enzymes as well as blood cortisol in dairy cows during the transition period

AST (U/L)	ALT (U/L)	Cortisol (ng/ml)	References
121	47.6	–	Sevinç et al. (1997)[a, *EL*]
93.08 ± 30.36	28.91 ± 11.84	–	González et al. (2011)[a, *EL*]
159.4 ± 8.9	–	–	Sentürk et al. (2010)[a, *EL*]
113.9	–	–	Filipejová and Kováčik (2009)[b, *EL*]
149 ± 40	22.86 ± 4.52	–	Xu et al. (2014)[a, *EL*]
–	–	29. 40 ± 16.13	Djoković et al. (2015)[b, *LG*]
–	–	14.14 ± 5.70	Youssef et al. (2016)[a, *EL*]

LG investigation during the late gestation time, *EL* investigation during the early lactation period

[a] Subclinical ketotic cows during transition phase

[b] Healthy transition cows

triglyceride, total cholesterol, albumin, and blood urea nitrogen were lower in early lactation than those in late pregnant and/or mid lactating cows (Djoković et al. 2015).

Conclusion

The data clearly demonstrate that dairy cows are vulnerable to the development of NEB and SCK during the transition period. Moreover, transitional dairy cows could succumb to both IR and oxidative stress; however, the exact role of IR in cows with SCK needs further investigation. It is, also, imperative to elaborate a potential nutritional strategy to facilitate an easy transition of cows through such a critical period and to minimize health problems and provide optimal productivity for the remainder of the lactation time.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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