Research in Veterinary Science 107 (2016) 246–256

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Dairy cows affected by ketosis show alterations in innate immunity and lipid and carbohydrate metabolism during the dry off period and postpartum

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ARTICLE INFO

Article history:
Received 8 December 2015
Received in revised form 17 May 2016
Accepted 18 June 2016

Keywords:
Dairy cows
Ketosis
Innate immunity
Blood variables
Milk production

ABSTRACT

The objective of this investigation was to search for alterations in blood variables related to innate immunity and carbohydrate and lipid metabolism during the transition period in cows affected by ketosis. One hundred multiparous Holstein dairy cows were involved in the study. Blood samples were collected at −8, −4, week of disease diagnosis (+1 to +3 weeks), and +4 weeks relative to parturition from 6 healthy cows (CON) and 6 cows with ketosis and were analyzed for serum variables. Results showed that cows with ketosis had greater concentrations of serum β-hydroxybutyric acid (BHBA), interleukin (IL)-6, tumor necrosis factor (TNF), serum amyloid A (SAA), and lactate in comparison with the CON animals. Serum concentrations of BHBA, IL-6, TNF, and lactate were greater starting at −8 and −4 weeks prior to parturition in cows with ketosis vs those of CON group. Cows with ketosis also had lower DMI and milk production vs CON cows. Milk fat also was lower in ketotic cows at diagnosis of disease. Cows affected by ketosis showed an activated innate immunity and altered carbohydrate and lipid metabolism several weeks prior to diagnosis of disease. Serum IL-6 and lactate were the strongest discriminators between ketosis cows and CON ones before the occurrence of ketosis, which might be useful as predictive biomarkers of the disease state.

1. Introduction

Ketosis is a common metabolic disorder of transition dairy cows during the early lactation period. It is important to note that ketosis first appears in a subclinical form, known as subclinical ketosis (SCK). In a smaller number of cows the disease progresses to a clinical form known as clinical ketosis (CK). Subclinical ketosis (SCK) is defined as an increase of ketone bodies (i.e. β-hydroxybutyric acid (BHBA), acetoacetate, and acetone) in the blood, urine, or milk, in the absence of obvious clinical symptoms. Almost 40% of dairy cows in North America have different degrees of SCK within a few weeks after calving, with the incidence varying widely and reaching as high as 80%, in some dairy herds (Duffield, 2000). On the other hand clinical ketosis (CK) is characterized by excess ketone bodies in blood, urine and milk, lack of appetite, lower milk production, rapid weight loss, and dry manure (Gordon et al., 2013a). The incidence of CK ranges between 2 and 15% in the first month of lactation (Gordon et al., 2013a).

The "gold standard" test for diagnosis of ketosis has been blood BHBA, which is the predominant circulating ketone body in ruminants and more stable in blood than the other two ketones, acetoacetate and acetone (Työppönen and Kauppinen, 1980). The generally used cut-off value for diagnosis of SCK is ≥1200 and up to 1400 μmol/L of blood BHBA (Suthar et al., 2013). Clinical ketosis is generally characterized by concentrations of BHBA in the blood >3000 μmol/L (Oetzel, 2007). Most dairy producers and veterinary practitioners focus more on diagnosis of ketosis and treatment of disease during the postpartum period, mostly disregarding the prepartum period.

Although much is known about the pathobiology of ketosis there is still a need to better understand both the etiology and pathogenesis of the disease. Ketosis has been correlated with a state of negative energy balance (NEB), which results from increased energy demand for milk production, especially during peak lactation (3–6 weeks postpartum) and relatively inadequate feed intake during this period of time (Çağdaş, 2013). All cows commonly undergo a period of NEB during peak lactation and excessive body fat mobilization, but not all animals experience hyperketonemia, and even less develop CK (1.5–4.0% of the herd) (Duffield et al., 1998; Oetzel, 2007). Mobilization of fatty

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http://dx.doi.org/10.1016/j.rvsc.2016.06.012
0034-5288/

acids from adipose is accompanied by elevated concentrations of non-esterified fatty acids (NEFA) in the blood circulation. During the state of NEB, gluconeogenesis is enhanced and a large portion of blood NEFA is converted into ketone bodies, as a different form of energy, in the liver hepatocytes (Gordon, 2013b). It was reported that it is not NEB itself, but inadequate metabolic adaptation that contributes to the development of ketosis (Herdt, 2000).

Several epidemiological studies have shown an association between ketosis and increased susceptibility to infectious diseases like mastitis and metritis (Erb and GroÈhn, 1988). Pro-inflammatory cytokines play a pivotal role in activating systemic inflammatory responses and acute phase proteins (APP) have been used as non-specific biomarkers of inflammation (Petersen et al., 2004; Dantzer and Kelley, 2007). In this study, we hypothesized that alterations in innate immunity reactants, as well as carbohydrate and lipid metabolism in the blood precede occurrence of ketosis. Therefore, the objectives of the current research were to evaluate perturbations of blood metabolites related to innate immunity as well as carbohydrate and lipid metabolism starting at −8 and −4 weeks prepartum. Specifically, concentrations of three serum metabolites: BHBA, non-esterified fatty acids (NEFA), and lactate; three major pro-inflammatory cytokines: interleukin-1 (IL-1); interleukin-6 (IL-6), and tumor necrosis factor (TNF); and two APP: haptoglobin (Hp) and serum amyloid A (SAA) were quantified at −8 and −4 weeks before parturition and during the week of disease diagnosis (+1 to +3 weeks) as well as at +4 weeks after calving.

2. Materials and methods

2.1. Animals and diets

One hundred pregnant Holstein dairy cows at the Dairy Research and Technology Centre, University of Alberta (Edmonton, AB, Canada) were screened and sampled in this study. All cows (i.e., 100 cows) were urine-tested with Ketostix strips (Bayer Corporation, Elkhart, IN) for presence of ketone bodies (i.e., urine acetoacetate) on weekly basis from −8 weeks prepartum to +8 weeks postpartum. Diagnosis of ketosis was based measuring of serum BHBA by Microplate reader kits (Sigma, St. Louis, MO, USA) at −8, −4, +1, +2, +3, and +4 weeks around calving, in all 100 cows. However, only 12 cows were selected and used in the study. Six pregnant multiparous (parity: ketosis 3.7 ± 0.8 vs healthy cows (CON) 3.0 ± 0.9; $P = 0.58$) cows were diagnosed postpartum with ketosis by urine Ketostix strips (Bayer Corporation, Elkhart, IN) and confirmed by a colorimetric method (i.e. serum BHBA ≥1400 μmol/L, kit (Ref. No. 2440-058) provided by Sigma, St. Louis, MO, USA) in the lab. All 6 cows with ketosis used in this study showed Ketostix test score greater than moderate (3920 μmoL of AcAc/L) and serum BHBA >1400 μmoL/L. All ketosis cows from the CON group had Ketostix test score lower than small (1470 μmol of AcAc/L) and serum BHBA lower than 1400 μmoL/L. Cows that had two or more diseases simultaneously (ketosis and another disease) were excluded from the ketotic group. Six clinically healthy cows that were homologous in age, parity, and body condition score (BCS at around 3 after calving) with the other 6 cows diagnosed with ketosis were selected as control (CON) group. All cows were clinically healthy before parturition. All experimental procedures were approved by the University of Alberta Animal Policy and Welfare Committee for Livestock, and animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993).

The experimental period lasted for 17 weeks from −8 weeks prepartum to +8 weeks postpartum (i.e. −8 to +8 weeks, 0 week means the week of parturition) for each cow. Dry matter intake (DMI) was calculated based on data collected from −8 to +8 weeks. Milk production was calculated from +1 to +8 weeks. Serum variables were analyzed from −8 weeks to +4 weeks. Milk compositions were determined on +2, +3, +5, and +7 weeks relative to parturition. All cows were offered a total mixed ration (TMR) during the experimental period (Tables 1 & 2). Feed was provided for ad libitum intake once daily at

Table 1
Prepartum diet for the dry off cows.

Item	Close-up diet (CUD)
Ingredient	% of DM
Alfalfa hay	10.0
Barley silage	60.0
CUD grain	30.0
Nutrient composition of CUD grain	% in 100 kg of mix
Ruminant TM Pak[a]	0.2775
Selenium 1000 mg/kg (UNscr FineCr)	0.2
Custom TM Complex Premix[b]	0.33
Vitamin A/D_3–1000-200[c]	0.006
Barley grain, rolled	39.5815
Flo-bond mycotoxin binder	0.5
Limestone	3.7
Magnesium chloride	1.645
Mag Ox-56%[d]	0.54
Scale molasses (60:40)	2.5
Canola meal	17.0
Vitamin E 50% Ads[e]	0.18
Soybean hulls, ground	33.0
Salt	0.54

[a] Ruminant TM Pak: a premix containing cobalt, copper, iodine, manganese, and zinc.
[b] Custom TM complex premix: a custom product supplying organic sources of cobalt, copper, manganese, and zinc.
[c] Vitamin A/D_3–1000-2003: Vitamin A acetate (retinyl acetate) and Vitamin D_3 (cholecalciferol).
[d] Mag Ox 56%: 56% magnesium guarantee.
[e] Vitamin E 50% Ads contains 226,800 IU of Vitamin E per pound.

Table 2
Ingredients of TMR fed to cows during early lactation.

Item	Early lactation diet
Ingredient % of DM	%, DM
Alfalfa hay	9.59
Barley silage	30.24
Alfalfa silage	9.64
High 16% dairy ration	50.53
Nutrient composition of dairy ration	% amount per kg
ADE Vit Pak-30 natural E[a]	0.05
Ruminant TM Pak[b]	0.11
Selenium, 1000 mg/kg (UNscr FineCr)	0.07
Custom TM Complex premix[c]	0.07
AminoShure - L[d]	0.33
Blood meal	3.50
Barley grain, rolled	39.90
Barley grain, ground	27.50
Di-calcium phosphate 21%	1.00
Vit D-10,000 KIU/kg	0.02
Diamond V XPC[e]	0.13
Dairy Xtract	0.02
Energizer RP10	2.75
Limestone	1.70
Mag Ox-56%[f]	0.43
Scale molasses (60:40)	1.25
Nutri A-Z C Dry	0.10
Amino plus (high bypass soy)[g]	8.00
Vitamin E 50% ads[h]	0.01
Soy bean meal-47.5%	1.25
Sodium bicarbonate	0.80
Salt	0.50
Poultry-tallow	0.50
Biotin 2%-Rovimix H-2[i]	0.01
Wheat distillers grain (50:50)	10.00

[a] ADE Vit Pak-30 Natural E: a premix containing vitamins A, D3, and E.
[b] Ruminant TM Pak: a premix containing cobalt, copper, iodine, manganese, and zinc.
[c] Custom TM complex premix: a custom product supplying organic sources of cobalt, copper, manganese, and zinc.
[d] AminoShure - L: hydrogenated vegetable oil, and L-lysine monohydrochloride (Halchemix, Port Perry, ON, Canada).
[e] Diamond V XPC: concentrated yeast (Diamond V Mills, Cedar Rapids, IA).
[f] Mag Ox 56%: 56% magnesium guarantee.
[g] Amino Plus: a high by-pass soy meal.
[h] Vitamin E 50% Ads contains 226,800 IU of Vitamin E per pound.
[i] DSM Nutritional Products (Parsippany, NJ).