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Bovine Subclinical Ketosis in Dairy Herds in Iran

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ABSTRACT

Subclinical ketosis (SCK) is defined as elevated concentrations of ketone bodies in the absence of clinical signs of ketosis. It is an important metabolic disease in dairy cattle during early lactation and is associated with losses in milk production and several other periparturient diseases. Limited information is available regarding the prevalence of SCK in dairy herds in Iran. The objectives of this study were (i) to determine the incidence of SCK in the dairy herds in Kerman province of Iran using serum β-hydroxybutyrate (BHB) concentrations, and (ii) to investigate the relationship between serum concentrations of BHB and glucose of cows with SCK. In the present study, 90 multiparous Holstein cows (4–6 years old) from 11 commercial dairy herds were evaluated 3–4 weeks after calving. The distribution of blood BHB concentrations seemed to suggest a cut-off point of 1200 μmol/L between cows with and without SCK. At this cut-off point, 14.4% of tested cows (13/90) were classified as subclinically ketotic, with the prevalence rate within herd ranging from 10% to 20%. Cows with SCK were detected in all the investigated dairies except one. Blood glucose concentrations in cows with SCK were significantly lower ($p \leq 0.05$) than in cows without SCK, and serum BHB and glucose concentration were inversely correlated ($r = -0.43$, $p \leq 0.05$). The results suggest that, using a cut-off of 1200 μmol/L, BHB concentrations can be used during early lactation for diagnosis and to make management decisions for prevention and treatment.

Keywords: subclinical ketosis, ketone body, β-hydroxybutyrate

Abbreviations: BHB, β-hydroxybutyrate; IQR, interquartile range; SCK, subclinical ketosis

INTRODUCTION

Subclinical ketosis (SCK) is defined as elevated concentrations of circulating ketone bodies in the absence of clinical signs of ketosis (Andersson, 1988). Clinical and subclinical ketosis both result in increased concentrations of ketone bodies in tissues and milk of the cows. The ketone bodies include acetone, acetoacetate, and β-hydroxybutyrate (BHB). β-Hydroxybutyrate is synthesized from absorbed butyrate in the rumen epithelium of ruminants and by the ketogenesis of the hepatocytes in the conversion of long-chain fatty acids from fat mobilization. Acetoacetate and BHB are freely distributed and transported in the blood, and seem interconvertible in various tissues (Bruss, 1997).

Subclinical ketosis is a common disease in high-producing dairy cows that is caused by a negative energy balance, and can affect milk production (Dohoo and Martin, 1984) and reproduction (Andersson and Emanuelson, 1985; Andersson, 1988; Whitaker *et al.*, 1993). It has been shown that SCK can be associated with an increased frequency of left-displaced

abomasa and other periparturient diseases (Geishauser *et al.*, 1997). Nonspecific immunity has been shown to be impaired in cows suffering from SKC (Sartorelli *et al.*, 2000). Overall prevalence of SCK has been reported to range from 6.9% to 14.1% of cows in the first two months of lactation (Dohoo and Martin, 1984; Andersson and Emanuelson, 1985; Nielen *et al.*, 1994; Duffield *et al.*, 1997); however, prevalence as high as 34% has been reported (Kauppinen, 1993; Duffield *et al.*, 1998). The peak prevalence of SCK occurs in the second or third week after calving (Geishauser *et al.*, 2000). Because of the economic consequences, the detection of cows with SCK at an earlier stage is important (Enjalbert *et al.*, 2001), and tests results could be used on a herd basis to determine the incidence of SCK and indicate the necessity for further investigations and management improvements (Carrier *et al.*, 2004).

The gold standard diagnostic test for SCK is the measurement of BHB in serum or plasma because of its stability (Duffield, 2000; Herdt, 2000). To distinguish between normal cows and cows with SCK, a cut-off point of blood BHB at 1200 μmol/L has been recommended by several investigators (Duffield *et al.*, 1997, 1998; Geishauser *et al.*, 1998; Jorritsma *et al.*, 1998).

Limited information is available regarding the prevalence of SCK in dairy cows in Iran. The objectives of this study were (i) to determine the incidence of SCK in the dairy herds in Kerman province of Iran using serum BHB concentrations, and (ii) to investigate the relationship between the concentrations of BHB and glucose in blood from cows with SCK.

MATERIALS AND METHODS

Sample collection

A total of 100 multiparous Holstein cows (4–6 years old) with high milk producing records (more than 9000 kg, 305-day milk production) were randomly selected from 11 commercial dairy herds that had a total of 3542 cows in Kerman province, Iran. Kerman is a semi-arid area located close to the south-central border of the largest desert area in the country, with cold winters and warm and droughty summers. The cows had two to five lactations, with body weight ranging from 550 to 610 kg. The animals were kept in free-stall housing and the calving season was February to March. Prepartum cows in the transition period (3–4 weeks before parturition) were housed in a separated dry lot. All diets were based on alfalfa, corn silage, and a combination of concentrate including corn, soya meal and bone meal. Health and fertility records were maintained on all herds by the dairymen and their veterinarians. Observations relating to disease of all cows were made by the production medicine veterinarian (senior author). Signs of clinical diseases including clinical ketosis, such as hard dry faeces, diminished appetite, decreased milk production and loss of body weight, and concurrent diseases around early lactation, such as abomasal displacements and traumatic reticuloperitonitis (TRP), were identified by physical examinations and by qualitative evaluation of urinary ketone bodies. Since the likely risk time for occurrence of ketosis is during 2–5 weeks after parturition and to avoid a likely peak in blood BHB after a meal, the samples were taken during 3–4 weeks after parturition and 4–5 hours after

feeding. Blood was drawn directly to the serum clot tube using a single jugular venepuncture and a Vacutainer needle. The samples were immediately transported to the laboratory in a cooler with ice packs and were processed within an hour of blood collection. The samples were centrifuged at 2000*g* for 20 min at 4°C, and serum was stored at −20°C until analysis. Six samples with visual haemolysis were excluded from the study. Owing to insufficient collection of serum from 4 cows in the original sampling, these cows were also excluded, resulting in a final sample of 90 cows.

Biochemical analysis

An automated chemistry analyser (VITALAB Selectra 2, Merck, Germany) was used to measure the serum BHB and glucose concentrations by BHB dehydrogenase (Ranbut, Randox, UK) and glucose oxidase (Kimia-Teb, Iran) assays, respectively (Bruss, 1997).

Statistical analysis

All statistical analyses were performed using Analyse-it software for Excel, version 1.71 (Analyse-it Software, Leeds, UK). The data was tested for normality using Kolmogorov–Smirnov test, which is a goodness-of-fit test for continuous data to determine whether a sample comes from a given hypothesized distribution. Neither serum BHB nor glucose concentrations were normally distributed; therefore, data from each parameter were expressed as median with interquartile range (IQR) and 25th–75th centile. For correlation (r) between serum BHB and glucose concentration, the Spearman rank test was used, which measures the strength of the associations between two random variables. The concentration of blood glucose between cows with and without SCK was compared using the Mann–Whitney test, which is the nonparametric equivalent of the independent-samples t-test and is used when the sample data are not normally distributed. The differences were considered significant at values of $p \leq 0.05$.

RESULTS

Ninety Holstein multiparous cows from 11 commercial dairy herds in Kerman province were tested for the prevalence of SCK. The calving seasons and management programmes were similar, and all cows were 4–6 years old with two to five lactations. No cow was excluded from this study owing to clinical disease. The frequency of distribution of BHB concentrations is shown in Figure 1. In this study, because of an apparent break in the distribution of blood BHB and in accordance with the cut-off point suggested by others (Duffield *et al.*, 1997, 1998; Geishauser *et al.*, 1998; Jorritsma *et al.*, 1998), cows with BHB concentrations higher than 1200 μmol/L were classified as having SCK. Overall, 14.4% of tested cows (13/90) were considered subclinically ketotic, with the prevalence rate within herd ranging from 10% to 20%. Table I shows the median, interquartile range (IQR) and 25th–75th centile for serum BHB and glucose concentrations in cows with and without SCK. Serum glucose concentrations in the subclinical ketotic cows were significantly ($p \leq 0.05$)

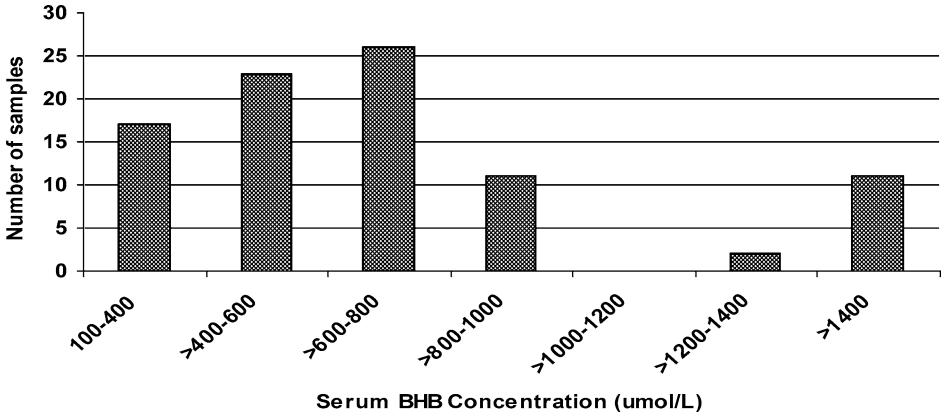

Figure 1. Frequency of distribution of serum β-hydroxybutyrate (BHB) concentrations, based on 90 samples taken from multiparous Holstein cows during 3–4 weeks after parturition and 4–5 hours after feeding. An apparent break in the distribution of blood BHB was present at >1000 to 1200 μmol/L

lower than in the cows without SCK. Cows with SCK were detected in all the investigated dairies except one (Table II). Figure 2 presents the correlation coefficient observed between serum concentrations of BHB and glucose. The concentrations of BHB and glucose in serum were significantly ($p \leq 0.05$) and inversely correlated ($r = -0.43$; -0.57 to -0.22 95% CI) in the tested cows.

DISCUSSION

Subclinical ketosis is an important metabolic disease in early-lactation dairy cattle that is associated with milk production losses and several periparturient diseases. Since high-

TABLE I
Serum β-hydroxybutyrate (BHB) and glucose concentrations in 90 multiparous Holstein cows

	Cows with BHB concentration <1200 μmol/L ($n = 77$)			Cows with BHB concentration >1200 μmol/L ($n = 13$)			All cows ($n = 90$)		
	Median	IQR[a]	25th–75th centile	Median	IQR	25th–75th centile	Median	IQR	25th–75th centile
BHB (μmol/L)	600	280	420–700	1620	310	1510–1820	620	355	455–810
Glucose (mg/dl)	50	6	48–54	42.0	6	40–46	50	7	46–53

[a]IQR, interquartile range.

TABLE II
Number and percentage of cows with subclinical ketosis (SCK)

Dairy no.	No. of samples with BHB concentration >1200 μmol/L	Percentage of cows with SCK
1 (15)[a]	3	20
2 (9)	1	11.1
3 (5)	1	20
4 (8)	1	12.5
5 (10)	2	20
6 (10)	1	10
7 (7)	1	14.2
8 (8)	1	12.5
9 (9)	1	11.1
10 (5)	1	20
11 (4)	0	0

[a]Numbers in parenthesis indicate the number of selected cows in each dairy.

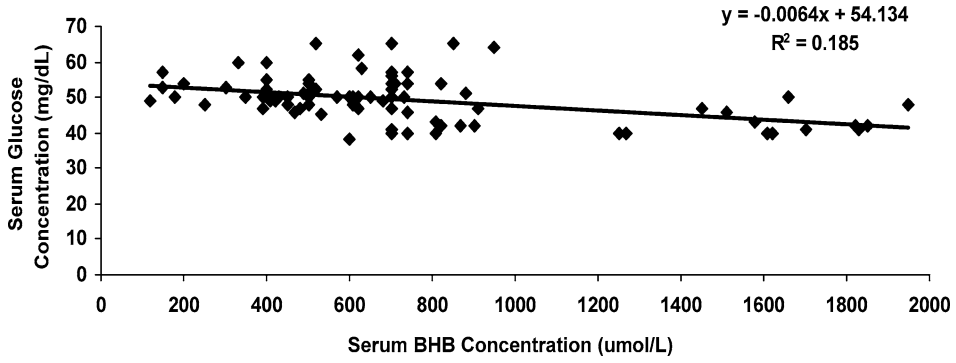

Figure 2. Correlation coefficient and best line fit between serum β-hydroxybutyrate (BHB) and glucose concentrations, based on 90 samples taken from multiparous Holstein cows during 3–4 weeks after parturition and 4–5 hours after feeding. Using the Spearman rank test, serum BHB and glucose concentrations were inversely correlated ($r = -0.43$; -0.57 to -0.22 95% CI; $p \leq 0.05$) in the tested cows

producing dairy cows have higher demands for glucose and greater mobilization of non-esterified fatty acids, which results in the elevation of ketone body synthesis, the incidence of SCK is likely to remain important in the dairy industry (Duffield, 2000). The costs of SCK vary from farm to farm and include both decreased milk production and increased occurrence of periparturient diseases and reproduction disorders (Duffield, 2000).

A variety of tests are available for SCK monitoring of dairy herds. Since BHB is the predominant circulating ketone body in SCK and is relatively stable in whole blood, plasma or serum, both *in vivo* and *in vitro* (Custer *et al.*, 1983; Dohoo and Martin, 1984), measurement of blood BHB concentrations as the gold standard method is the most accurate test for herd monitoring (Oetzel, 2004). It has been suggested that testing a herd for the prevalence of SCK via blood BHB sampling in early lactation would be useful in almost any dairy herd, particularly for investigating herds with presumptive SCK (Oetzel, 2004). It has been shown that among all ketone bodies, BHB has the most marked diurnal variations in relation to feed intake, with peak levels about 4 h post feeding (Whitaker *et al.*, 1983; Duffield, 2000); therefore, in this study blood samples were taken at fixed times. We measured BHB in serum because it has been reported that BHB measured with an enzymatic method in plasma is generally lower than that measured in serum, possibly because of interference from anticoagulants (Custer *et al.*, 1983). The presence of haemolysis in serum has been shown to interfere with BHB analysis, causing elevated measurements (Duffield *et al.*, 1998); we therefore excluded samples with haemolysis.

Subclinical ketosis may start at serum BHB concentrations above 1000 μmol/L and clinical ketosis at about 2600 μmol/L; however, at exactly what level individual cows will express clinical signs is extremely variable (Andersson, 1984). Studies using blood BHB concentrations for assessing SCK report a range of values from 1000 μmol/L to 1400 μmol/L for defining a subclinical threshold (Whitaker *et al.*, 1983, 1993); however, the distribution of blood BHB concentrations in our study seemed to suggest a cut-off point of 1200 μmol/L between cows with and without SCK. In our study, 14.4% of cows had serum BHB concentrations higher than the cut-off point. Using the same threshold, previous studies have reported 16.4% of positive cows in Ontario, Canada (Geishauser *et al.*, 1998) and 14.0% in The Netherlands (Jorritsma *et al.*, 1998). The prevalence of SCK increases from primiparous to multiparous cows (Detilleux *et al.*, 1994), and is highest during lactation weeks 2 and 3 (Duffield *et al.*, 1998; Geishauser *et al.*, 1998). Several other factors influence the prevalence of hyperketonaemia, including age (Dohoo and Martin, 1984; Andersson, 1988), season (Whitaker *et al.*, 1993), and breed (Andersson, 1988).

The significant negative correlation coefficient between BHB and glucose concentrations observed in the present study is in accordance with the fact that hypoglycaemia is the driving force in bovine subclinical and clinical ketosis that ultimately causes ketonaemia (Bruss, 1997). Plasma BHB concentrations combined with plasma glucose values were used to classify cows as having poor energy status (Whitaker *et al.*, 1983). However, in SCK dairy cattle can become ketonaemic without the presence of significant hypoglycaemia (Grohn *et al.*, 1983).

In conclusion, the results of this study show that the prevalence of SCK in Kerman province is considerable and is in close agreement with the prevalence reported by others (Dohoo and Martin, 1984; Geishauser *et al.*, 1998; Jorritsma *et al.*, 1998). Furthermore, these results suggest that, using a cut-off of 1200 μmol/L, BHB concentrations can be used during early lactation for diagnosis of SCK. To prevent the economic loss due to this disease, early treatment of positive cows is necessary and prevention of the disease has to be achieved through proper nutritional programmes for the dry and early-lactation cows.

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