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Article *in* Asian Journal of Animal and Veterinary Advances · April 2013

DOI: 10.3923/ajava.2013.604.612

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Asian Journal of Animal and Veterinary Advances 8 (4): 604-612, 2013
ISSN 1683-9919 / DOI: 10.3923/ajava.2013.604.612

Prevalence of Ketosis and its Correlation with Lactation Stage, Parity and Peak of Milk Yield in Iran

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ABSTRACT

Ketosis is a metabolic disorder which usually occurs in cows during early lactation in both, industrialized and developing countries. There are no data available about the prevalence of ketosis in major dairy producing provinces in Iran. This study investigated the prevalence of ketosis among 1,002 Iranian Holstein cows from days 5 to 50 post calving in various parity and lactation stages in 13 regions of Iran. Whole blood beta-Hydroxybutyrate (BHBA) concentration equal or more than 1,400 and 3,000 μmol L^{-1} were considered as subclinical ketosis (SCK) and clinical ketosis, respectively. The prevalence of SCK and clinical ketosis were 13.9 and 3.4%, respectively and higher prevalence of ketosis during second (42.2%) and third weeks (24.8%) post calving. The ketosis prevalence was found significant between regions, but not parity. Prevalence of ketosis was higher in Gorgan, Shahrekord and Sari than the other regions. The negative correlations between prevalence of ketosis (BHBA = 1400 μmol L^{-1}) and blood glucose concentration and lactation stage were significant. In addition, the positive correlation between blood glucose concentration and lactation stage was also significant. Blood glucose and BHBA concentrations were not correlated with the parity stage. The mean milk production for cows suffering from clinical ketosis, SCK and healthy cows were 28, 35 and 45 kg day^{-1}, respectively. Significant negative correlation coefficients were found between blood BHBA with peak milk yield. In conclusion, the present results show that the best times to identify clinical and SCK are 14 and 17 days after calving.

Key words: Beta-hydroxybutyric acid, subclinical ketosis, clinical ketosis, dairy cows

INTRODUCTION

Bovine ketosis typically occurs in early lactation and is caused by the combination of appetite limitation and the high demand of the mammary gland for nutrients, particularly glucose. The insufficient supply of glucose may lead to decrease in carbohydrate status, to decrease in insulin

secretion, to increase in fat mobilization and finally to increase in hepatic ketogenesis (Baird, 1982). Clinical and subclinical ketosis (SCK) both result in increased concentrations of ketone bodies in cows tissues and milk (Enjalbert *et al*., 2001).

Measuring blood BHBA has been reported as the gold standard test for detecting ketosis (Oetzel, 2007). This ketone body is more stable in blood than the other ketone bodies i.e. acetone or acetoacetate (Tyopponen and Kauppinen, 1980). Moreover, cows at the early lactation stage with blood BHBA concentrations above the cut-off point of 1,400 μmol L^{-1} are three folds more at risk to develop displaced abomasums or clinical ketosis. Blood BHBA concentrations of above 1,940 μmol L^{-1} would further suppress the animals' heath resulting in reduced milk yield (Duffield *et al*., 1997). Consequently, in the herds with a high incidence of ketosis, the periods between calving, first and last services were found to be longer than those of the normal cows (Melendez, 2006). Therefore, the economic losses from ketosis include treatment costs of clinically ill cows, lost milk production, increased days open and culling. These stand at an estimated cost of approximately USD 145 per case (Guard, 1994).

Ketosis occurs in dairy herds in both, industrialized and developing countries. Sato *et al*. (2005) reported 34.7% of SCK and 15.3% clinical ketosis in a study of 150 dairy cows in Japan. In a different study, the prevalence of mild and severe ketonemia was 22 and 3.8%, respectively, in Jordanian dairy cattle (Al-Rawashdeh, 1999), while Zilaitis *et al*. (2007) reported that 57.7% of cows had SCK in Lithuania. In Iran, Sakha *et al*. (2007) using 1,200 μmol BHBA/L blood cut-off point, reported 14.4% of the tested cows (13 out of 90 cows) were sub-clinically ketotic in the Kerman province, with the prevalence rate within the studied herd ranging from 10 to 20%. However, Kerman province is not a major dairy producing province in Iran, carrying only about 3.3% of the total population of dairy cows in the country. In addition, the average daily milk yield in Kerman province is approximately 25 L/cow, lower than many other provinces, such as 33.5 and 30.5 L cow day^{-1} in Isfahan and Khorasan provinces, respectively.

The aims of this study were firstly to investigate the prevalence of ketosis (subclinical and clinical) in 13 dairy producing regions in Iran and secondly to investigate the relationship between ketosis with parity (number of calving), lactation stage (days in milk) and peak milk yield in the studied regions.

MATERIALS AND METHODS

Study population: One thousand and two Holstein cows with high milk production (more than average milk yield of the respective herd) records were randomly selected (10 to 25 cows per herd) from 57 commercial dairy farms (each consisting of between 150 to 3,000 heads) in 13 regions in Iran. All farms selected for this study were equipped with auto-feeder, feed mixer, milking machine and computer system with management software to record the health status and production records of individual cows. The average milk production in 57 farms during 305 days of lactation ranged between 9,000 to 11,000 kg with an average 3.5% fat and 3% protein.

Data on stage of lactation, parity and milk yield of each experimental cow were provided by the respective farm. Highest milk yield recorded for each cow within day 40 to 80 was considered as its peak milk yield.

Blood sampling and analysis: Blood samples were taken via the coccygeal vein from the experimental cows between day 5 to day 50 post-parturition (Oetzel, 2007) and each whole blood sample was analyzed for BHBA content using Precision Xtra **β**-ketone test strip (Abbott Park, Illinois). This was achieved by dipping the sensor directly onto the surface of the blood-filled tube immediately after collection. The "Precision Xtra **β**-ketone" monitoring system is a simple and direct

electrochemical test (Iwersen *et al.*, 2009), in which the ketone test strip containing enzyme β-hydroxybutyrate dehydrogenase, which oxidizes BHBA to acetoacetate. This reduces NAD+to NADH and is then reoxidized to NAD+ by an electron transfer mediator molecule (Oetzel and McGuirk, 2009), giving an output reading in μmol L^{-1} BHBA. Cows with blood BHBA concentration equals to/or above 1,400 μmol L^{-1} were considered to suffer from SCK while those equal to/or over 3,000 μmol L^{-1} were considered as clinically ketotic (Oetzel, 2004). Blood glucose concentration was measured using glucose test strip (Abbott Park, Illinois).

Statistical analysis: Descriptive statistics of the data were presented with correlation matrix (R). The variable BHBA values were transformed to natural logarithms in order to achieve normality. Therefore, both blood BHBA (nlBHBA) and glucose concentrations followed normal distribution and they were used in the analysis as continues variables. Correlation coefficients (r) between parity, lactation stage, blood BHBA and glucose concentration at different times were estimated and tested. Analysis of covariance (ANCOVA) was implemented to consider the effect of region, parity, stage of lactation, blood glucose and peak milk yield on nlBHBA (model: nlBHBA = region+parity+stage of lactation+blood glucose+peak milk yield+error). All calculations were performed using Proc GLM (SAS, 2003).

RESULTS

A total of 1,002 blood samples from 57 commercial dairy farms in 13 regions in Iran with mean lactation stage and parity of 21 day and 2.6 were evaluated for their BHBA levels, glucose contents, parity, lactation stage and peak milk yield. The mean of BHBA level for all the cows studied was 763 μmol L^{-1} with a range of 100 to 4,900 μmol L^{-1} while the mean of glucose level was 53 mg dL^{-1} with a range of 30 to 111 mg dL^{-1} (Table 1). The mean value for peak milk yield for all the cows studied was 42.57 kg day^{-1} with 28, 35 and 45 kg day^{-1}, respectively, for the clinical ketosis, SCK and healthy groups (Table 1).

The mean BHBA and glucose levels for cows with clinical ketosis were 3,597 μmol L^{-1} and 35 mg dL^{-1}, respectively and the mean lactation stage and parity for cows with clinical ketosis was 14 d and 2.7, respectively (Table 2). As for the cows with SCK, the mean BHBA, glucose levels, lactation stage and parity were 1,780 μmol L^{-1}, 48 mg dL^{-1}, 17 d and 2.8, respectively, while the above-mentioned values for the healthy cows were 477 μmol L^{-1}, 54 mg dL^{-1}, 22 d and 2.6, respectively (Table 2). The concentration of BHBA in cows with clinical ketosis (3597 μmol L^{-1}) was significantly higher than that of the normal cows (477 μmol L^{-1}) and cows with SCK

Table 1: Descriptive statistics for number of parity, lactation stage, blood glucose and BHBA and peak milk yield

Variables	Mean	Standard deviation	Minimum	Maximum
Parity (n)	2.64	1.587	1	12
Lactation stage (d)	20.91	12.236	5	80
Glucose (mg dL^{-1})	52.91	9.948	30	111
BHBA (μmol L^{-1})	763.27	0.788	100	4900
Peak milk yield (kg)	42.57	8.578	14	64

Table 2: Mean±SE blood BHBA and glucose, lactation stage, parity and peak milk yield of normal and ketotic cows

	BHBA (μmol L^{-1})	Glucose (mg dL^{-1})	Lactation stage (day)	Parity (n)	Peak milk yield (kg)
Normal	477±8^{c}	54±0.30^{a}	22±0.44^{a}	2.60±0.06^{a}	45±0.26^{a}
SCK	1780±37^{b}	48±0.90^{b}	17±0.90^{b}	2.80±0.13^{a}	35±0.51^{b}
Clinical	3597±92^{a}	35±1.20^{c}	14±1.00^{b}	2.70±0.24^{a}	28±0.97^{c}

Means in the same column with different superscripts differ ($p<0.05$)

Table 3: Prevalence of ketosis (SCK and clinical ketosis) in different regions of Iran

Regions	Farm (n)	Cows (n)	Ketosis (%)	SCK (%)	Clinical ketosis (%)
Arak	2	41	9.75	7.30	2.45
Qazvin	3	58	12.00	8.60	3.40
Gorgan	4	68	31.00	23.65	7.35
Isfahan	10	206	15.00	12.60	2.40
Karaj	7	99	11.11	9.00	2.11
Mashhad	4	42	9.50	7.10	2.40
Rey	10	174	21.26	16.09	5.17
Sari	2	42	28.60	19.04	9.56
Semnan	1	13	9.00	0.00	9.00
Shahrekord	1	20	30.00	30.00	0.00
Shiraz	1	13	0.00	0.00	0.00
Tabriz	1	21	9.50	9.50	0.00
Varamin	11	205	17.80	15.90	1.90
Mean		77	15.73	12.21	3.52

(1780 μmol L^{-1}) ($p<0.05$; Table 2). In addition, the concentration of BHBA in cows with SCK (1780 μmol L^{-1}) was significantly higher than that of the healthy cows (477 μmol L^{-1}) ($p<0.05$). Glucose concentrations in healthy cows (54 mg dL^{-1}) were significantly higher than that of the cows with SCK (48 mg dL^{-1}) and clinical ketosis (35 mg dL^{-1}) ($p<0.05$; Table 2). Peak milk production in healthy cows (45 kg) was also significantly higher than that of the cows with SCK (35 kg) and clinical ketosis (28 kg) ($p<0.05$; Table 2). The mean parity variations observed in the herds for all cows regardless of their health status was not significant. The best time to identify clinical and SCK was 14 and 17 days after calving (Table 2).

The overall prevalence of ketosis recorded in this study was 15.73, with 12.21% of the cows classified as SCK and 3.52% as clinical ketosis (Table 3). The prevalence of ketosis was higher during the second and third weeks after calving at 42.2 and 24.8%, respectively. The prevalence of ketosis in the present study, based on the cut-off point of BHBA = 1,400 μmol L^{-1} blood was the highest in Gorgan (31%), ShahrKord (30%) and Sari (28.6%) regions and the lowest in Semnan, Tabriz and Mashhad (9 to 9.5%) (Table 3).

There were no differences between the various parities. However, there was a difference ($p<0.01$) between regions and peak milk yield (Table 4). Increasing lactation stage and blood glucose was associated with decreased ($p<0.01$) nlBHB in the ANCOVA (Table 4). Lactation stage, blood glucose concentration and peak milk yield affected by blood BHBA concentration significantly ($p<0.01$; Table 4).

Higher numbers of blood sample were taken from Varamin, Isfahan and Rey regions where the larger number of industrial dairy farms located in those regions. The highest whole blood BHBA concentrations were recorded in farms situated in Sari (1,145 μmol L^{-1}) and Gorgan (1,056 μmol L^{-1}), while the lowest belonged to the farms located in Shiraz (377 μmol L^{-1}) and Tabriz (571 μmol L^{-1}). The highest blood glucose concentration was recorded for the farms located in Rey (55 mg dL^{-1}), Tabriz (56 mg dL^{-1}) and Mashhad (56 mg dL^{-1}) regions while the lowest belonged to the farms in Semnan (46 mg dL^{-1}), ShahrKord (49 mg dL^{-1}) and Sari (49 mg dL^{-1}) (Table 5).

The highest parity values were recorded in Tabriz (3.1), Isfahan (3.0) and Mashhad (2.7) regions and the lowest was in Arak region (1.8). The lowest and highest values for lactation stage

Table 4: Influence of region, parity, lactation stage and blood glucose on the natural logarithm of the blood BHBA in 1,002 cows from 13 regions of Iran

Source of variation	Sum of squares	df	F-value	p-value
Region	25.23	12	4.67	<0.001
Parity	2.03	2	2.26	0.11
Lactation stage	5.11	1	11.35	<0.001
Blood glucose	29.03	1	64.44	<0.001
Peak milk yield	80.54	1	178.79	<0.001
Error	443.27	984		

Table 5: Mean (± SE) of blood BHBA and glucose, lactation stage, parity and peak milk yield of Holstein cows at the post calving stage from 13 regions of Iran

Regions	BHBA (μmol L^{-1})	Glucose (mmol L^{-1})	Lactation stage (day)	Parity (n)	Peak milk yield (kg)
Arak	602±118^{cd}	53±1.20^{ab}	24±1.80^{ab}	1.8±0.16^{b}	39±1.00^{d}
Qazvin	730±87^{abcd}	54±1.40^{ab}	20±1.20^{abc}	2.6±0.19^{ab}	50±1.00^{a}
Gorgan	1056±119^{ab}	52±1.60^{ab}	21.0±1.30^{abc}	2.4±0.15^{ab}	37±1.00^{d}
Isfahan	654±44^{b}	52±0.70^{ab}	20.0±0.90^{abc}	3.0±0.12^{a}	47±0.60^{ab}
Karaj	688±62^{bcd}	54±0.70^{ab}	26.0±2.00^{a}	2.6±0.15^{ab}	41±0.70^{cd}
Mashhad	671±95^{bcd}	56±2.00^{a}	22.0±1.60^{abc}	2.7±0.30^{a}	44±1.00^{bc}
Rey	851±67^{abc}	55±0.70^{a}	22.0±0.70^{abc}	2.6±0.13^{ab}	40±0.70^{d}
Sari	1145±154^{a}	49±1.10^{bc}	10.0±0.50^{d}	2.7±0.18^{ab}	45±1.40^{bc}
Semnan	890±387^{abc}	46±1.60^{c}	18.0±2.20^{bc}	2.2±0.40^{ab}	41±1.60^{cd}
Shahrekord	926±164^{abc}	49±2.80^{bc}	16.0±1.60^{cd}	2.6±0.34^{ab}	46±2.00^{ab}
Shiraz	377±56^{d}	51±2.20^{ab}	17.5±2.00^{bc}	2.5±0.45^{ab}	40±1.20^{d}
Tabriz	571±121^{cd}	56±2.20^{a}	22.0±2.50^{abc}	3.1±0.47^{a}	47±1.70^{ab}
Varamin	743±45^{abcd}	53±0.60^{ab}	21.0±0.70^{abc}	2.5±0.12^{ab}	40±0.52^{d}

Means in the same column with different superscripts differ ($p<0.05$)

Table 6: Pearson correlation coefficients between blood BHBA and glucose with parity and lactation stage during two months post calving (n= 1,002)

	Parity	Lactation stage	Glucose	BHBA	Peak milk yield
Parity	1				
Lactation stage	-0.046				
P†	0.144	1			
Glucose	0.056	0.145*			
P	-0.072	0.0001	1		
BHBA	-0.003	-0.154*	-0.311*		
P	0.919	0.0001	0.0001	1	
Peak milk yield	0.019	0.013	0.178*	-0.415*	
P	0.530	0.664	0.0001	0.0001	1

†Prob>|r| under H_0: r = 0, *Significant at $p<0.05$, ** Significant at $p<0.01$

were recorded in Sari (10.0 day) and Karaj (26.0 day), respectively (Table 5). The highest peak milk yield was recorded for the farms located in Ghazvin (50 kg day^{-1}), Isfahan (47 kg day^{-1}) and Tabriz (47 kg day^{-1}), while the lowest were in the farms located in Arak (39 kg day^{-1}) and Gorgan (37 kg day^{-1}) (Table 5).

Significant ($p<0.01$) negative correlation coefficients were found between the blood BHBA with lactation stage (r = -0.154) and blood glucose (r = -0.311) (Table 6). The correlation coefficients between blood glucose concentration and lactation stage were positive (r = 0.145; $p<0.01$). The blood

glucose and BHBA concentration had significant relationships with peak milk yield ($r = 0.178$ and $r = -0.415$ respectively; $p<0.01$) but not with parity ($r = -0.056$ and $-r = 0.003$ respectively; $p>0.05$) (Table 6).

DISCUSSION

The most commonly used cut-off point for SCK is ≥1400 μmol L^{-1} (14.4 mg dL^{-1}) of blood BHBA while a much higher level (≥3000 μmol L^{-1}) is used to denote clinical ketosis (Oetzel, 2004 and 2007). Duffield and LeBlanc (2009) also suggested that the best threshold for predicting subsequent risk of clinical ketosis based on the serum sample obtained during the first two weeks postpartum was 1400 μmol L^{-1} of BHBA. The Precision Xtra **β**-ketone test has been reported to be a useful cow-side ketone test for detection of SCK in postpartum dairy cows. Using whole blood sample and a cut-off value of ≥1400 μmol BHBA/L of blood, the Precision Xtra **β**-ketone test has been proven to achieve excellent test characteristics and high diagnostic performance (Iwersen *et al*., 2009; Kupczynski and Cupok, 2007).

Using the same test procedure in this study, 15.73% of the tested cows from 13 regions in Iran were identified to suffer from ketosis with majority of the incidences classified as SCK (12.21%) and the remaining of 3.52% were clinically ketotic. The rate of SCK recorded in this study was nearly identical to the 15.4% reported by Sakha *et al*. (2007) for Kerman province using 1,200 μmol BHBA/L blood cut-off point. However, an earlier study by Pourjafar and Heidari (2003) using milk BHBA in 511 Holstein cows reported a more than two-fold higher SCK prevalence (38%) in Khorasan province of Iran. Oetzel (2007) investigated 1,047 cows in 74 herds in Wisconsin and reported an overall ketosis prevalence of 15.7% with only 26% of the herds investigated showing the ketosis prevalence of below 10% (a cut-off point considered as an alarming level for herd-based ketosis testing).

In a recent study conducted in Ontario veterinary college, Duffield and LeBlanc (2009) reported that 24 out of 136 (17.6%) transitionally raised cows had BHBA concentrations ≥1,400 μmol L^{-1} of serum in the first week post-calving. Similarly a survey of 60 herds conducted by Cornell University reported that, in 40% of the herds, more than 15% of the cows sampled had serum BHBA concentrations >,200 μmol L^{-1} (Ospina *et al*., 2010). These findings well indicate that ketosis is a widely prevalent metabolic disease in dairy farms including those in the industrialised countries.

There was a high negative correlation observed between blood BHBA and lactation stage ($r = -0.154$) in this study, with higher prevalence of ketosis during the second (42.2%) and third weeks (24.8%) post calving. The results obtained are in agreement with those of Dohoo and Martin (1984) who reported higher quantities of milk ketone bodies in the first month as compared to the second month of lactation and observed that the peak prevalence of hyperketonemia occurred in the third and fourth weeks of lactation. Duffield (2000) and Geishauser *et al*. (2000) also reported that during the same period, approximately 40% of all the cows studied were at least once affected by SCK, although the incidence and prevalence were highest in the first and second weeks after parturition. Duffield (2000) reported that the median days from calving to diagnose clinical ketosis and displaced abomasum was around 11 days and therefore, an efficient SCK monitoring program should focus on the first 2 to 3 wk of lactation. A more recent study in Iran reported a SCK prevalence rate of 7.2% with its peak occurring during the fourth week of lactation (Haghighat-Jahromi and Nahid, 2011).

Among the 13 regions studied, only Arak, Mashhad, Semnan, Shiraz and Tabriz regions recorded the prevalence rate of below 10%, indicating that 8 out of the 13 regions studied had higher than the 10% prevalence, suggested as the alarming level for herd-based ketosis testing (Oetzel, 2007).

There was a highly negative correlation observed between blood BHBA and glucose concentrations (r = -0.311) in this study but a positive correlation was found between blood glucose concentration and lactation stage (r = 0.145). It should be noted that when the limiting factor is the supply of glucose precursors, blood ketone concentration will climb up to very high levels while blood glucose concentration remains very low (Oetzel, 2007). The net result of these changes and imbalances is that during the periods of negative carbohydrate and energy balances, ruminants normally have moderately elevated blood ketone body concentrations and moderately depressed blood glucose concentrations (Herdt, 2000).

The present results showed that the whole blood BHBA in ketotic cows increased significantly, whereas glucose concentrations decreased as compared to that of the healthy cows. The results of this study are in agreement with those of Oetzel and McGuirk (2009), who reported that blood glucose, was consistently low in cows at the time of their first ketosis diagnosis, even if the cows were in very early lactation and apparently had type II ketosis with underlying fatty liver.

After calving, the initiation of milk synthesis and the rapidly increasing milk production greatly increased demand for glucose for milk lactose synthesis, while feed intake has not reached its maximum yet. Limited feed intake during the early postpartum period means that the supply of propionate for glucose synthesis is also limited and therefore, an increase in fat mobilization occurs (Drackley *et al*., 2001). Several authors Rajala-Schultz and Grohn (1999); Geishauser *et al*. (2000) reported the negative effect of clinical ketosis on milk production with an average production loss of as high as 25% or 353.4 kg per lactation (LeBlanc *et al*., 2005). The mean daily peak milk yield for clinical ketotic, subclinical ketotic and healthy cows in this study were 28, 35 and 45 kg, respectively, suggesting a decline ranging from 22 to 38%, compared to the healthy cows.

CONCLUSION

Results of this study revealed that the prevalence rate of ketosis differed among the 13 regions of Iran; averaged 15.73%, with 12.21% SCK and 3.52% clinical ketosis. These results are in agreement with those previously reported in different countries, suggesting that ketosis is a widely prevalent metabolic disorder among dairy cows. The results also suggested that the prevalence of ketosis reached its highest rate in the second and third weeks after calving and significant negative correlations were found between blood BHBA and glucose concentration; and between blood BHBA concentration and peak milk yield.

ACKNOWLEDGMENT

The authors would like to thank Dr Pejman Ajilchi, General Manager of Sana Dam Pars Company (Tehran, Iran) for financial support of this study.

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