

## EVALUATION OF BETA-HYDROXY BUTYRATE AND GLUCOSE IN SUBCLINICAL KETOSIS IN INDUSTRIAL HERDS OF HOLSTEIN COWS

Amoughli Tabrizi, B.<sup>1</sup>, Safi, S.<sup>2</sup>, Asri Rezaee, S.<sup>3</sup>, Hassanpour, A.<sup>1</sup> and Mousavi, G.<sup>1</sup>

<sup>1</sup> Department of Clinical Sciences, Veterinary Faculty, Islamic Azad University, Tabriz Branch, Tabriz, Iran; <sup>2</sup> Department of Clinical Sciences, Faculty of Specialized Veterinary Sciences, Islamic Azad University, Science & Research campus, Tehran, Iran; <sup>3</sup> Department of Clinical Sciences, Veterinary faculty, Uremia University

### SUMMARY

Subclinical Ketosis (SCK) is one of the most prevalent metabolically disorders found commonly in dairy farms worldwide which is caused by lack of balance in diet and energetic deficiency in animals. The objective of this study was to study of BHB and glucose levels in healthy Holstein cows and cows with SCK and to determine of the prevalence of the disease, using BHB level in blood serum as the gold standard. In this study 7 dairy farms were chosen in Shahriar, (Tehran province, Iran). Samples were taken from 100 cows at two periods: 1) last week of pregnancy (dry period), 2) 1, 2, 4 and 8 weeks after parturition. Serum samples were harvested and BHB levels were measured, using RANBUT kits (Randox, England) and glucose levels was measured by commercial available kits (Ziest Chimi, Iran) using spectrophotometer. In this study, the prevalence of SCK, using 1.2, 1.4 and 1.7 mmol/L BHB as the cut-off point were calculated as 18% and 14% and 4%, respectively. In this study the mean levels of BHB, in two-month-post parturition group was higher than the cows at their last week of pregnancy. Mean glucose levels in cows at two months after parturition and also in cows with SCK were lower than in cows at their last week of pregnancy and healthy cows at two months after parturition.

There was a significant correlation coefficient ( $r = -0.27$ ,  $P < 0.05$ ) between BHB and glucose levels in cows at their last week of pregnancy. Correlation coefficient analysis also showed a relationship between BHB concentration and glucose levels ( $r = -0.64$ ,  $P < 0.05$ ) in cows at their second months after pregnancy. The correlation between BHB and glucose levels in cows affected by SCK was not significant ( $P > 0.05$ ). There was a relationship between BHB and glucose levels ( $r = -0.53$ ,  $P < 0.05$ ) in the healthy cows at second month after pregnancy.

**Keywords:** subclinical ketosis, glucose, BHB, cattle

### INTRODUCTION

Subclinical ketosis is the accumulation of large quantities of ketone bodies in blood and tissues. Ketone bodies include  $\beta$ - Hydroxy butyric acid, acetoacetic acid and acetone. The maintenance of adequate concentrations of glucose in blood is critical to the regulation of energy metabolism. In ruminants carbohydrates are fermented in the rumen to fatty acids principally acetate, propionate and butyrate. Propionate and amino acids are the major precursors for gluconeogenesis with glycerol and lactate of lesser importance (1, 5, 18).

The initial event in the pathogenesis of ketosis is negative energy balance and the accompanying mobilization of non esterified fatty acids from adipose tissue. Negative energy balance is prevalent in dairy cows during the first 2 to 8 weeks of lactation since feed intake doesn't keep pace with the rapid increase in energy demands for milk production. Ketosis may be clinical or subclinical and effected milk production and reduced reproduction (6, 7,8).

The economic impact of ketosis is derived from treatment costs, reduced milk production and reduced fertility. The disease is seldom fatal, so death loss isn't an important economic factor (9, 10).

Clinical ketosis is frequently associated with concurrent disease both infectious and metabolic. In many cases, ketosis occur secondary to another disease. In other instances, ketosis may be the initial disease (18, 19).

Clinical ketosis cause gastrointestinal and nervous sings. SCK often is without clinical sings and cause drop in milk production, reduced fertility and partial anorexia that result in less body condition. Diagnosis of SCK is important for prevention of economic losses (11, 12, and 14). The objective of this study was to study the BHB and glucose levels in healthy Holstein cows and cows with SCK and to determine the prevalence of SCK, using BHB levels in blood serum as the gold standard.

## MATERIAL AND METHODS

In this survey 7 dairy farms were chosen in Shahriar, Tehran province, Iran. Samples were taken from 100 cows at two periods: 1) last week of pregnancy (dry period), and 2) 1, 2, 4 and 8 weeks after parturition. Blood samples were taken from jugular veins and serum was harvested by 3000 rpm centrifuge, for 10 min. BHB levels were measured using RANBUT kits (Randox. England) and glucose levels were measured by commercial kits (Ziest Chimi, Iran) using spectrophotometer (Biowave F 2100) (20,21).

## STATISTICAL ANALYSIS

Paired student's t-Test was used to evaluate the differences between groups. Simple linear correlation was used to find the relationships between the variables, using SPSS 10 for Windows.

## RESULTS

In this study, the prevalence of SCK using 1.2, 1.4, and 1.7 mmol/L BHB, as the cut-off points for detection of SCK, calculated as 18% and 14% and 4%, respectively at two months after parturition. The results are shown in table 1. Results of the biochemical blood tests are shown in Table 2.

**Table 1.** The prevalence of subclinical ketosis at two months after parturition

Prevalence of subclinical ketosis prevalence	BHB (1.2 mmol/L)	BHB (1.4mmol/L)	BHB (1.7 mmol/L)
	18%	14%	4%

**Table 2.** BHB and glucose mean levels ( $\pm$  SD) in cows after and before parturition and in cows with sub clinical ketosis and healthy

Time	1) Healthy cows before parturition	2) Healthy Cows after parturition	3) Sub clinical ketosis cows	4) Healthy cows
BHB mmol/L	0.48 $\pm$ 0.22 <sup>2</sup>	0/61 $\pm$ 0/52 <sup>1</sup>	1/67 $\pm$ 0/12 <sup>4</sup>	0/44 $\pm$ 0/31 <sup>3</sup>
Glucose mg/L	49/78 $\pm$ 11/28 <sup>2</sup>	42/78 $\pm$ 17/34 <sup>1</sup>	23/14 $\pm$ 4/31 <sup>4</sup>	45/98 $\pm$ 16/54 <sup>3</sup>

\* (1–4) Means within a row with common superscript differ significantly ( $P < 0.05$ )

## DISCUSSION

SCK (also called acetoanaemia) occurs in higher yielding cows in early lactation. Acetone is produced by the liver and released into the blood where it acts as an intoxicant to the cow. The disease is caused by an inadequate intake of “starchy” foods in a cow, which is already mobilizing body fat. SCK is a disease of dairy cattle and is prevalent in most countries where intensive farming is practiced. The occurrence of the disease is very much dependent upon management and nutrition. One of the energy metabolism parameters monitored in this study was blood glucose concentration. Statistically significant differences between the two groups of dairy cows (before and after parturition) and between healthy and SCK cows were found ( $P < 0.05$ ).

The mean level of glucose in cows at two months after parturition and also in cows with SCK was lower than the cows in their last week of pregnancy and healthy cows in two month after parturition. Decrease in blood glucose concentrations reported in response to fat supplementation in the first stage of lactation in dairy cows. Our results are in accordance with the results of other studies (5, 13, 15, 16, 17, and 23). Glucose is a substance that plays a fundamental role in all animals. In the last weeks of fetal development, the fetus uses around 46% of maternal glucose taken up by the uterus. Additionally, a cow producing 30 kg of milk per day uses at least 2 kg of blood glucose to synthesize lactose for milk. The end of pregnancy and the beginning of lactation, therefore, represent a time when there is a massive increase in need for glucose. This poses an enormous challenge for the liver that has to synthesize all of this glucose from propionate and amino acids as well as a challenge for other tissues and organs that have to adapt to a reduction of glucose availability. Glucose is an equally important energy source for the ovary and the reduced glucose availability in the beginning of lactation can negatively impact the reestablishment of ovarian activity after calving (2, 3).

Another parameter of energy metabolism monitored was the blood concentration of BHB. Compared with glucose, BHB is a more sensitive indicator of energy metabolism disruptions, and its concentrations are increased by lipid mobilization. In our study, BHB concentrations in the SCK group at week 8 post partum were higher than in healthy groups and BHB concentrations in

cows after parturition was higher than the cows before parturition ( $P < 0.05$ ). Our results were similar with other studies (4, 5, 6, 13, 16, 17, 22, and 23).

There was a significant relationship ( $r = -0.27$ ,  $P < 0.05$ ) between BHB and glucose levels at last week of pregnancy. There was also a significant relationship ( $r = -0.64$ ,  $P < 0.05$ ) between BHB and glucose levels in their second month after pregnancy.

No significant relationship was found between BHB and glucose levels in cows affected by SCK ( $P > 0.05$ ).

Correlation coefficient analysis in the healthy cows at second month after pregnancy showed a relationship between BHB and glucose levels ( $r = -0.53$ ,  $P < 0.05$ ).

## REFERENCES

- 1) Ballard, C. Mandevu, S. P. Sniffen, C. J. Emanuele S. M. Carter, M. P., (2001): Effect of feeding an energy supplement to dairy cows pre- and postpartum on intake, milk yield, and incidence of ketosis, *Animal Feed Science and Technology*, Volume 93, Issues 1–2, Pages 55–69.
- 2) Bell, A.W. (1995): Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *J. Anim. Sci.* 73: 2804.
- 3) Bell, A.W. 1996. The transition cow: actualized homeorrhexis. Proc. 58th Cornell Nutrition Conference for Feed Manufacturers. Cornell University, Ithaca, NY.
- 4) Dann, H.M. Drackley, J.K. Morin, D.E., (2002): Effects of prepartum feed intake and postpartum health disorders on dairy cow performance and blood and liver constituents. IlliniDairyNet. <http://www.traill.uiuc.edu/preferences>
- 5) Duffield, T. F., (2004): Monitoring strategies for metabolic disease in transition dairy cows. 23rd World Buiatrics Congress. Quebec, Canada. July 11–16.
- 6) Duffield, T. F., (2006): Minimizing subclinical metabolic diseases in dairy cows. *WCDS Advances in Dairy Technology*. 18: 43–55.
- 7) Enjalbert, F. Nicot, M.C. Bayourthe, C. Moncoulon, R., (2001): Ketone bodies in milk and blood of dairy cows: relationship between concentrations and utilization for detection of subclinical ketosis, *Journal of Dairy Science*, Volume 84, Issue 3, Pages 583–589.
- 8) Geishauser, T. Leslie, K. Tenhag, J. Bashiri, A., (2001): Evaluation of eight cow-side ketone tests in milk for detection of subclinical ketosis in dairy cows, *J Dairy Sci.* 83: 296–299
- 9) Gerloff, B J., (2000): Dry cow management for the prevention of ketosis and fatty liver in dairy cows, *The Veterinary Clinics of North America. Equine Practice*, Volume 16, Issue 2, Pages 283–292.
- 10) Gillund, P. Reksen, O. Gröhn, Y. T. Karlberg, K., (2001): Body condition related to ketosis and reproductive performance in Norwegian dairy cows, *Journal of Dairy Science*, Volume 84, Issue 6, Pages 1390–1396.
- 11) Gutzwiller, A., (1998): Determination of beta-hydroxybutyrate in milk using test strips: a new aid for the diagnosis of subclinical and clinical ketosis in the cow, *Schweizer Archiv Fur Tierheilkunde*, Vol 140, Issue 3, Pages 120–124.
- 12) Itoh, N; Koiwa, M; Hatsugaya, A; Yokota, H; Taniyama, H; Okada, H; Kudo, K., (1998): Comparative analysis of blood chemical values in primary ketosis and abomasal displacement in cows, *Zentralblatt Fur Veterinarmedizin. Reihe A*, Volume 45, Issue 5, Pages 293–298.
- 13) Kaczmarowski, M. Malinowski, E. Markiewicz, H., (2006): Some hormonal and biochemical blood indices cows with retained placenta and puerperal metritis. *Bull Vet Inst Pulawy*. 50: 89–92.
- 14) Mandevu, P. Ballard, C. S. Sniffen, C. J. Tsang, D. S. Valdez, F. Miyoshi S. Schlatter, L., (2003): Effect of feeding an energy supplement prepartum and postpartum on milk yield and composition, and incidence of ketosis in dairy cows, *Animal Feed Science and Technology*, Volume 105, Issues 1–4, Pages 81–93.

- 15) Nafikov, R. A. Ametaj, B. N. Bobe, G. Koehler, K. J. Young, J. W. Beitz, D. C. (2006): Prevention of fatty liver in transition dairy cows by subcutaneous injections of Glucagon. *J. Dairy Sci.* 89: 1533–1545.
- 16) Oikawa, S. Oetzel, G.R. (2006): Decreased insulin response in dairy cows following a four-day Fast to induce hepatic lipidosi. *Dairy Sci.* 89: 2999–3005.
- 17) Padilla, L. Shibano, K. Inoue, J. Matsui, T. Yano, H., (2005): Plasma vitamin C concentration is not related to the incidence of ketosis in dairy cows during the early lactation period. *J Vet Med Sci.* 67(9): 883–6.
- 18) Radosaitis, o. m. Gay, C.C., Blood, D.C., and Hinchcliff, K.W., (2000): *Veterinary Medicine*, Saunders, pages 1452–1462.
- 19) Reist, M. Erdin, D. K. Euw, D. Tschümperlin, K. M. Leuenberger, H. Hammon, H. M. Künzi, N. Blum, J. W., (2003): Use of threshold serum and milk ketone concentrations to identify risk for ketosis and endometritis in high-yielding dairy cows, *American Journal of Veterinary Research*, Volume 64, Issue, Pages 188–194.
- 20) Stockhom, S. L. Scott, M. A., (2002): *Fundamentals of Veterinary Clinical Pathology*. PP: 9, 44, 317–318, 356, 435–439, 443–451, 524–528.
- 21) Tietz, N., (2000): *Text Book of Clinical Chemistry* Tietz. PP: 784–795, 807–810.
- 22) Yalcin, A. Polat, Ü. Çetin, M., (2002): The Relations between some biochemical blood parameters and milk yield during various lactation stages in High-Yielding dairy cows. *J.Fac.Vet. Med.* 21: 65–69.
- 23) Zadnik, T., (2003): A Comparative study of the Hemato-Biochemical parameters between clinically healthy cows and cows with displacement of the Abomasums. *Acta Veterinaria (Beograd)*. 53(5–6): 297–309.