Comparative study of the sera concentration of prolactin and cortisol hormones in healthy cows and the cows with subclinical ketosis

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Abstract: Understanding the pathogenesis and serum parameters of ketosis leads to proper diagnosis and choose of accurate preventional procedures. In this study 200 blood samples were collected through jugular vein from cows were at the pick of the production, randomly (2 months after parturition). Then serum separated and serumic values of the betahydroxibutyric acid (BHBA) were measured by Randox kit. The cows with BHBA >1.4 mmol/l, were considered subclinical ketosis group and cows that had BHBA <1.4 mmol/l were taken at normal group. There after the levels of prolactin and cortisol in the serum were measured using biochemical kits by ELISA in both groups. 14% of cows had subclinical ketosis. Results suggested that the cortisol and prolactin values in the serum of the in the subclincal ketosis group were higher than normal group and the difference of the means of these parameters were significant between the groups ($p<0.05$). There were significant correlation between BHBA and glucose with prolactin levels in the serum ($r= 0.84$, $p<0.01$ and $r= 0.73$, $p<0.01$ respectively). The correlation between levels of BHBA and glucose in serum with blood concentration of the cortisol were significant ($r= 0.81$, $p<0.01$ and $r= 0.51$, $p<0.05$ respectively). On the bases of the results obtained it is concluded that the increasing of sera concentrations of cortisol and prolactin hormones can confirm and prognose the subclinical ketosis in the dairy cows.
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1. Introduction

Subclinical ketosis (SCK) 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 (21, 25). 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 affected milk production and reduced reproduction (7, 8 and 16). 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. 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 (21, 25).

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 (25, 28). With understanding the pathogenesis of this metabolic disorder we can find an approach for diagnosis and prevention of this disease, so we can prevent economic losses of that (17, 28). In this study the levels of prolactin and cortisol hormones in serum of the normal and cows with subclinical ketosis compared.

2. Material and Methods

200 cows postafter 2 months of parturition were randomly selected from 7 dairy farms throughout the east Azarbaijn provine in Iran. Blood samples were taken from jugular veins and serum was harvested by centrifuging blood samples by 3000 rpm 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). Cows that had BHBA >1.4 mmol/l considered in subclinical ketosis group and cows with BHBA <1.4 mmol/l assigned into normal group (6). The prolactin and cortisol levels of serum were measured by with biochemical kits in both groups.

Significant difference between the groups evaluated by 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 SPSS15 soft ware.

3. Results

In this study, the prevalence of SCK, considering 1.4 mmol/l of BHB, as the cut-off point for detection of SCK, was 14% at two months after parturition. The results are shown in Table 1. Mean values of BHBA and blood glucose levels are shown in Table 2.

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

Prevalence of subclinical ketosis	SCK	normal
	28(14%)	172(86%)

Table 2) BHB and blood glucose values (± SD) in the cows under this study

	No.	Mean	SD
BHB (mmol/l)	200	0.61	0.52
Glucose(mg/dl)	200	42.78	17.34

Values of the blood biochemical parameters including BHBA, glucose, prolactin and cortisol in the normal and SCK groups are shown in Tables 3-6. Mean differences between the groups were statistically significant.

Table 3) BHB mean levels (± SD) in the normal and SCK cows

	No.	Mean(mmol/l)	SD	significant
SCK group	28	1.68	0.12	*
Normal group	172	0.45	0.31	

*: The difference between means was significant (P<0.05).

Table 4) Glucose mean levels (± SD) in the normal and SCK cows

	No.	Mean(mg/dl)	SD	significant
SCK group	28	23.14	4.31	*
Normal group	172	46.00	16.54	

*: The difference between means was significant (P<0.05).

Table 5) Prolactin mean levels (± SD) in the normal and SCK cows

	No.	Mean(ng/ml)	SD	significant
SCK group	28	16.57	1.55	*
Normal group	172	11.73	0.27	

*: The difference between means was significant (P<0.05).

Table 6) Cortisol mean levels (± SD) in the normal and SCK cows

	No.	Mean(ng/ml)	SD	significant
SCK group	28	102.78	10.76	*
Normal group	172	58.61	2.50	

*: The difference between means was significant (P<0.05).

The correlation of these parameters was observed in the table 7. The correlation between BHBA and glucose is negative and significant (r= -0.64 and p<0.01). A positive and significant correlation was observed between BHBA and prolactin and cortisol (p<0.1 in the both, r= 0.84 and r= 0.81, respectively). The correlations between glucose and these hormones were significant and positive, too (r=0.73, p<0.01 and r=0.51, p<0.05 respectively).

Table7: The correlation between biochemical parameters in the cows

The coorelation between parameters	Regression index(r)	Significant
BHBA and Glucose	- 0.64	**
BHBA and Cortisol	0.81	**
BHBA and Prolactin	0.84	**
Glucose and Cortisol	0.51	*
Glucose and Prolactin	0.73	**

*: The difference between means was significant (P<0.05).

**: The difference between means was significant (P<0.01).

4. Discussion:

SCK (also called acetonaemia) 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(7,8 and 23). In this study cows that had BHBA >1.4 mmol/l were taken at subclinical ketosis group and cows that had BHBA <1.4 mmol/l were taken at normal group (6).

One of the energy metabolism parameters monitored in this study was blood glucose concentration. Statistically significant difference between the two groups of dairy cows (normal and SCK) was found (P < 0.05). The mean level of glucose in cows with SCK was lower than the healthy cows in two months 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 (4,5,11 and 30). 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 period can negatively impact the reestablishment of ovarian activity after calving (15,23 and 24).

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 were higher than in healthy groups ($P < 0.05$). Our results were similar with other studies (12, 17, 18 and 20,).

There was a negative significant correlation between BHB and glucose levels in the serum in these cows ($r = - 0.64$ and $P < 0.01$). In the other studies this result were observed, too (9, 15, 19, 22, 26, 27, 29 and 30).

The serum's values of the cortisol and prolactin in the subclincal ketosis group were high than normal group and the difference of the means of these parameters were significant between two groups ($p<0.05$, both). The mean levels of the cortisol in serum in the cows with SCK were 102/57±11.13 and in the normal cows was 59.69±17.41 ng/ml. The correlation between levels of BHBA and glucose in serum with blood concentration of the cortisol were significant ($r= 0.81$, $p<0.01$ and $r= 0.51$, $p<0.05$ respectively) (table 7). The parturition and lactation are stress for cow and induce the secretion of the glucocorticoids. Cortisol is one of the glucocorticoids(3,6). Other surveys are showed that with ketosis or other disorders the concentration of the cortisol in blood increase in the dairy cows (1,10,11 and 12).

The mean serum levels of the prolactin in the cows with SCK were 16.57±4.40 and in the normal cows was 11.73±1.76 ng/ml. The correlation between levels of BHBA and glucose in serum with blood concentration of the prolactin were significant ($r= 0.84$, $p<0.01$ and $r= 0.73$, $p<0.01$ respectively) (table 7). After parturition active the mammary gland and increase the secretion of the prolactin hormone. In the cows with high production the secretion of this hormone and the incidence of SCK is high (2,13,14 and 31). The results of this study suggest that the serum levels of the prolactin is high in cows with subclinical ketosis in comparison with healthy cows.

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