INCIDENCE, EARLY DIAGNOSIS OF SUBCLINICAL KETOSIS AND DETERMINATION OF LIVER DYSFUNCTIONS IN COWS IN BURSA REGION*

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SUMMARY

Two hundred fortyfour cows and heifers in Bursa Region were divided into four groups in prepartum and postpartum periods in this study. Total leucocyte counts, haematocrit values, serum ß Hydroxybutyrate concentrations and level of keton bodies in milk and/or urine were determined in all groups. Incidence of subclinical ketosis was found as 16.39 % and the highest incidence was observed within the first month of lactation (28.81 %). Also, the incidence in prepartum period was found as 16.21 %.

Serum glucose, GLDH, AST, ALT, ALP, GGT, LDH, total bilirubin, direct bilirubin, indirect biliribin, total protein, albumin and globulin levels were estimated in 20 cows with ß Hydroxybutyrate concentrations less than 1.00 mmol/l and 45 cows with ß Hydroxybutyrate concentrations higher than 1.00 mmol/l. Serum ß Hydroxybutyrate concentrations, GLDH, LDH, total bilirubin, indirect bilirubin ($p<0.001$), direct bilirubin and albumin levels ($p<0.05$) were found statistically different in cows with clinical ketosis compared with controls. Serum ß Hydroxybutyrate concentrations in ketosis group were positively correlated with serum GLDH, AST, ($p<0.001$), total bilirubin and indirect bilirubin ($p<0.01$) levels.

As a result it was concluded that economic losses can be minimized by early diagnosis of ketosis by estimating serum ß Hydroxybutyrate

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* *"Bursa Çevresindeki İneklerde Subklinik Ketozisin İnsidensi, Erken Tanısı ve Karaciğer Fonksiyon Bozukluklarının Değerlendirilmesi" başlıklı doktora tezinin özetidir.*

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concentrations periodically during the last month of gestation and within three months after calving in large farms with high yielding dairy cows.

Key Words: Subclinical ketosis, ß Hydroxybutyrate, Dairy cows.

ÖZET

Bursa Çevresindeki İneklerde Subklinik Ketozisin İnsidensi, Erken Tanısı ve Karaciğer Fonksiyon Bozukluklarının Değerlendirilmesi

Bu çalışmada Bursa çevresinde prepartum ve postpartum dönemlerde olan ikiyüzkırkdört inek ve düve dört guruba ayrıldı. Tüm guruplarda total lökosit sayısı, hematokrit değer, serum ß Hidroksibutirat konsantrasyonu, idrar ve/veya sütte keton cisimcikleri düzeyi belirlendi. Subklinik ketozis insidensi % 16.39 olarak bulundu ve en yüksek insidensin laktasyonun birinci ayı (% 28.81) içerisinde olduğu gözlendi. Ayrıca, prepartum dönemde insidens % 16.21 olarak bulundu.

ß Hidroksibutirat konsantrasyonu 1.00 mmol/l'den düşük 20 ve ß Hidroksibutirat konsantrasyonu 1.00. mmol/l'den yüksek 45 inekte serum glukoz, GLDH, AST, ALT, ALP, GGT, LDH, total bilirubin, direkt bilirubin, indirekt bilirubin, total protein, albumin ve globulin düzeyleri belirlendi. Kontrol gurubuyla karşılaştırıldığında klinik ketozisli ineklerde serum ß Hidroksibutirat konsantrasyonları, GLDH, LDH, total bilirubin, indirekt bilirubin ($p<0.001$), direkt bilirubin ve albumin ($p<0.05$) düzeylerinde istatistiki farklılıklar bulundu. Ketozis gurubunda serum ß Hidroksibutirat konsantrasyonu ile AST, LDH ($p<0.001$), total bilirubin, direkt bilirubin ($p<0.01$) düzeyleri arasında pozitif bir korelasyon saptandı.

Sonuç olarak, yüksek verimli süt ineklerinin bulunduğu işletmelerde doğumdan bir ay önce ve doğumdan üç ay sonraki dönem içerisinde belirli aralıklarla serum ß Hidroksibutirat konsantrasyonunun ölçülüp hastalığın erken dönemde belirlenmesi ile ekonomik kayıpların azaltılabileceği görüşüne varılmıştır.

Anahtar Kelimeler: Subklinik ketozis, ß Hidoksibutirat, Süt inekleri.

INTRODUCTION

Ketosis is a metabolic disease of high yielding dairy cows characterized by formation of excessive keton bodies in blood, urine and milk due to impaired carbonhydrate and volatile fatty acid metabolism[1]. High yielding dairy cows reach 40 % of total lactation within 12 weeks after calving[2], and if energy requirements for milk yield and maintenance can not

be met in this period, ketosis will develope[3]. It is reported that the disease also occurs before parturition[4]. The subclinical form of ketosis is more common than clinical form[2,5]. The diagnosis of subclinical form in which there is no clinical sign other than decreased milk production is important to prevent economic loses[6]. Metabolic profile test and determination of keton bodies in blood, urine and milk, and blood glucose level is used to show the energy status of dairy cows[2,7-9]. Determination of serum ß Hydroxybutyrate (BHOB) which is the highest concentration keton body, better reflects-the energy status[10-12]. Whitaker et all[13] have shown that energy deficiency in subclinical ketosis can be best determined by estimating serum ß Hydroxybutyrate concentration.

It is reported that it is important to determine the degree of liver damage in evaluating the severity of disease[3,14-18].

In this study, it was aimed to diagnose ketosis in early stage by estimating serum ß Hydroxybutyrate concentrations to determine its incidence in cows both kept on semi intensive farms and owned by peasants in Bursa region in Turkey.

MATERIALS and METHODS

Two hundred fortyfour cows and heifers were used as materials. Of these cows and heifers 81 ones were from two large semi-intensive farms and 163 ones were owned by peasants from 65 different stables in 23 villages in Bursa region.

The material in prepartum and postpartum period was divided into four groups to determine the incidence of subclinical ketosis.

Group I : 74 cows and heifers within the last month of pregnancy

Group II : 59 cows within the first month after parturition

Group III : 55 cows within the second month after parturition

Group IV : 56 cows within the third month after parturition

Blood, urine and/or milk samples were collected from each animal and white blood cell (WBC) counts, packed cell volume (PCV %), ß Hydroxybutyrate concentrations and level of keton bodies in milk/urine were determined.

Twenty cows with ß Hydroxybutyrate concentrations less than 1.00 mmol/l served as control group, 40 cows with ß Hydroxybutyrate concentrations higher than 1.00 mmol/l as subclinical ketosis group and 5 cows with clinical signs and ß Hydroxybutyrate concentrations higher than 1.50 mmol/l as clinical ketosis group.

WBC counts and PCV values were determined by routine procedures[19]. The sera were kept at -20°C and ß Hydroxybutyrate concentrations were estimated by commercial kits (Ranbut, Randox Co., Ireland). Glucose, GLDH, AST, ALP, GGT, LDH, total bilirubin, direct bilirubin, indirect bilirubin, total protein, albumin and globulin levels were determined by commercial kits from all samples in control, subclinical and clinical ketosis groups.

Analysis of variance was used to evaluate the significance of the differences between the mean values observed in different groups of the cattle.

RESULTS

In this research it was observed that all the cows except 5 ones (four primer, one seconder ketosis) were healthy.

Subclinical ketosis incidence in groups I, II, III and IV were 16.21 %, 28.81 %, 10.90 % and 8.92 %, respectively. Subclinical ketosis was diagnosed in 40 (16.39 %) and clinical ketosis was diagnosed in 5 (2.05 %) of 244 cows.

The results of ß Hydroxybutyrate concentration, glucose, GLDH, AST, ALT, ALP, GGT, LDH, total bilirubin, direct bilirubin, indirect bilirubin, total protein, albumin, globulin levels in cows with subclinical and clinical ketosis (45 cows) in prepartum and postpartum periods and in controls are shown in Table I. Statistical differences were observed in PCV, ß Hydroxybutyrate concentrations ($p<0.001$), glucose, total bilirubin ($p<0.01$), AST, ALP and LDH levels ($p<0.05$).

Statistical results of cows in control, subclinical and clinical ketosis groups are shown in Table II. As seen in Table II statistically differences were observed in ß Hydroxybutyrate concentrations in subclinical and clinical ketosis groups ($p<0.001$). GLDH, AST, LDH, total bilirubin, indirect bilirubin levels were statistically different ($p<0.001$) in clinical ketosis group, ALT levels ($p<0.01$) in subclinical ketosis group, direct bilirubin and albumin levels ($p<0.05$) in clinical ketosis group.

The mean ß Hydroxybutyrate concentrations in 244 cows and heifers in prepartum and postpartum periods are shown in Graphic I. The highest mean concentration was estimated within the first month after parturition (0.85 mmol/l).

Table: I

Mean Values ± sd of Some Blood and Serum Parameters in Control Group and in Cows With Subclinical and Clinical Ketosis in Prepartum and Postpartum Periods.

Parameters	Control Group	Group I	Group II	Group III	Group IV
WBC (mm^3)	6610.00±611.00	6933.33±829.78	6326.09±300.15	7133.33±554.37	5444.44±459.80
PCV (%)	23.90±0.65^{b}	22.91±0.98^{b}	27.17±0.88^{a}	26.16±2.07^{ab}	22.00±0.70^{b***}
BHOB (mmol/l)	0.20±0.02^{a}	1.41±0.08^{b}	1.65±0.14^{b}	1.61±0.18^{b}	1.31±0.11^{b***}
Glucose (mg/dl)	37.30±3.30^{a}	23.33±3.55^{ab}	33.54±3.66^{ab}	26.17±5.20^{ab}	20.11±5.61^{b**}
GLDH (U/l)	8.86±1.30	14.85±1.27	28.56±8.49	17.09±1.85	16.41±3.26
AST (U/l)	86.35±3.50^{b}	129.33±3.46^{ab}	144.50±10.24^{a}	107.08±5.55^{ab}	122.44±5.72^{ab*}
ALT (U/l)	23.20±1.40	29.75±1.28	28.18±2.19	26.54±2.79	31.55±2.36
ALP (U/l)	39.50±3.50^{b}	57.75±10.28^{a}	39.27±1.79^{b}	53.18±7.49^{ab}	37.56±3.51^{ab*}
GGT (U/l)	17.45±1.20	23.75±2.40	18.36±2.08	18.63±1.87	18.77±1.73
LDH (U/l)	721.00±27.00^{b}	890.80±42.75^{ab}	911.20±73.17^{a}	794.70±29.69^{ab}	909.10±27.23^{ab*}
T.Bilirubin (mg/dl)	0.25±0.04^{b}	0.46±0.03^{a}	0.40±0.05^{ab}	0.33±0.03^{ab}	0.40±0.08^{ab**}
D.Bilirubin (mg/dl)	0.08±0.01	0.17±0.02	0.15±0.03	0.10±0.02	0.17±0.04
I.Bilirubin (mg/dl)	0.16±0.03	0.29±0.02	0.16±0.03	0.21±0.01	0.20±0.05
T.Protein (mg/dl)	7.73±0.15	7.59±0.13	7.89±0.13	7.78±0.35	8.11±0.21
Albumin (mg/dl)	3.71±0.08	3.81±0.06	3.75±0.07	3.66±0.13	3.78±0.08
Globulin (mg/dl)	3.97±0.15	3.72±0.15	4.18±0.17	4.17±0.27	4.42±0.18

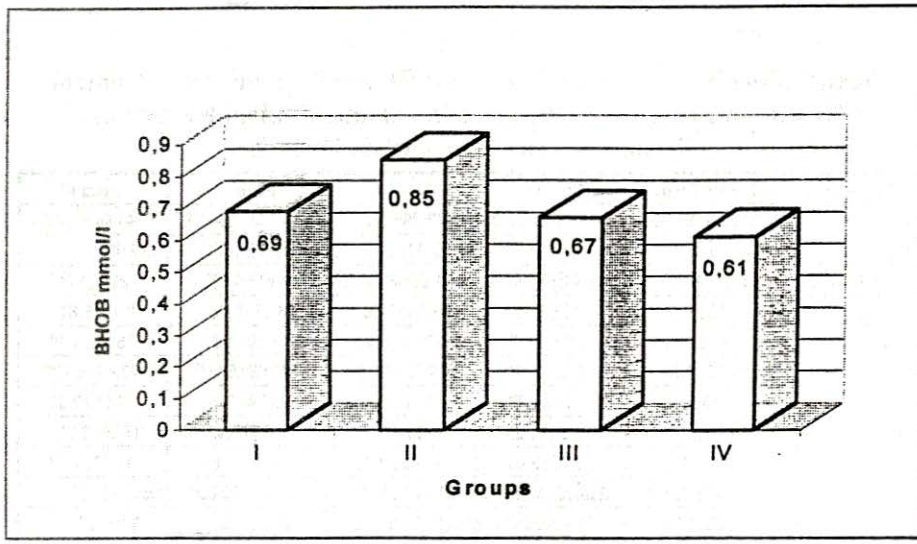
*p < 0.05 **p < 0.01 ***p < 0.001

Table: II

Mean Values ± sd of Some Serum Parameters in Control, Subclinical and Clinical Ketosis Groups.

Parameters	Control Group	Subclinical Ketosis	Clinical Ketosis Group
WBC (mm^3)	6610.00±611.00	6533.00±271.65	6120.00±947.64
PCV (%)	23.90±0.65	24.86±0.67	28.80±2.57
BHOB (mmol/l)	0.20±0.02^{c}	1.38±0.03^{b}	3.19±0.18^{a***}
Glucose (mg/dl)	37.30±3.30	27.88±2.41	26.00±7.19
GLDH (U/l)	8.86±1.30^{b}	15.88±1.07^{b}	78.20±33.67^{a***}
AST (U/l)	86.35±3.50^{b}	117.46±3.76^{b}	259.80±88.35^{a***}
ALT (U/l)	23.20±1.40^{b}	29.26±1.04^{a}	23.80±7.65^{ab**}
ALP (U/l)	39.50±3.50	46.71±3.30	39.45±4.22
GGT (U/l)	17.45±1.20	19.20±1.11	24.40±5.26
LDH (U/l)	721.00±27.00^{b}	853.00±17.85^{b}	1164.00±299.09^{a***}
T.Bilirubin mg/dl)	0.25±0.04^{b}	0.38±0.02^{b}	0.70±0.19^{a***}
D.Bilirubin mg/dl)	0.08±0.01^{b}	0.14±0.01^{ab}	0.24±0.09^{a*}
I.Bilirubin (mg/dl)	0.16±0.03^{b}	0.24±0.01^{b}	0.50±0.09^{a***}
T.Protein (mg/dl)	7.73±0.15	7.78±0.10	8.04±0.36
Albumin (mg/dl)	3.71±0.08^{ab}	3.79±0.04^{a}	3.36±0.16^{b*}
Globulin (mg/dl)	3.97±0.15	4.06±0.10	4.75±0.43

*p < 0.05 **p < 0.01 ***p < 0.001



Graphic: I

The Mean ß Hydroxybutyrate Concentrations in Groups, I, II, III and IV in Prepartum and Postpartum Periods.

DISCUSSION

Several studies[4,20-22] were performed to diagnose ketosis in early stages in dairy cows, but in these studies the incidence of the disease in cows owned by the peasants was not determined because they included large semi-intensive farms.

In this study the determined incidence of subclinical ketosis (16.39 %) was lower than those of reported by Şeker and Ünsüren[21], because the milk yield of cows owned by the peasants was less than those of in semi-intensive farms. Dohoo and Martin[4] found that the incidence of subclinical ketosis in cows within 65 days after parturition was 20.17 %. This result is similar to those of ours in the same period.

Although it is known that subclinical ketosis occurs most commonly in the period within 2-4 weeks after calving, in the present study the incidence of subclinical ketosis within the last month of pregnancy (16.21 %) shows that the disease is also important in the prepartum period. This may be supported by West[18] who showed that the fatty infiltration in liver in the period 2 weeks before calving reached the same levels as those in the period 2 weeks after calving.

The mean ß Hydroxybutyrate concentrations within the last month of pregnancy, first, second and third month after calving were 0.695, 0.855, 0.670 and 0.616 mmol/l, respectively. These results are similar with those of Andrea and others[23] who found that the mean ß Hydroxybutyrate concentrations in 30 days before calving, 10, 30 and 45 days after calving were 0.733, 0.990, 1.018 and 0.823 mmol/l, respectively. In both studies it was observed that the risk of disease increased in the period within one month after calving. Ghörn and others[15] showed that the mean ß Hydroxybutyrate concentration was the highest within the fourth week after calving.

The mean ß Hydroxybutyrate concentration in subclinical ketosis group was estimated as 1.380 mmol/l while it was 3.190 mmol/l in clinical ketosis group. Veenheusen and others[17] found that the mean ß Hydroxybutyrate concentration was increased 8.4 fold in cows with experimentally induced clinical ketosis.

Although it is reported that serum glucose level decreased while ß Hydroxybutyrate concentration is increased[23], in this study glucose concentration decreased only in group IV ($p<0.01$) compared with control group. Herd and others[10] showed that estimation of serum glucose level was not of value as much as serum ß Hydroxybutyrate concentration in diagnosis of subclinical ketosis in the postpartum period.

There is fatty infiltration and degeneration in hepatic tissues in ketosis[17]. Estimation of GLDH enzyme activity which is liver spesific in ruminants is of great value in diagnosis of liver damage in ruminants. GLDH levels increased significantly ($p<0.001$) in clinical ketosis group. According to these results it is shown that GLDH activity is a good indicator of liver damage in ruminants as it is reported by other researchers[24-27].

AST enzyme activity increased in group II ($p<0.05$) compared with controls. AST levels together with GLDH levels increased in clinical ketosis group ($p<0.001$). The results are in general agreement with those of other researchers[3,15,24]. Moreover, West[18] has showned that AST and GLDH levels increased in hepatic lipidosis.

In the present study it was found that ALP levels increased in the prepartum period. It may be related with the wide distribution of ALP activity in the body. Morever, it is probable that increase in the prepartum period may be related with plecental origin[28]. No significant difference was observed in GGT levels. Kauppinen[3] reported that ketosis does not cause either cholestasis or bile duct damage, since no significant increase in GGT levels is observed. Estimation of only LDH level is not enough to determine liver damage unless it is interpreted in relation to GLDH and AST[26]. Elevated LDH levels together with GLDH and AST levels in clinical ketosis group showed liver damage and supported the other researchers[18,26].

Serum bilirubin levels increase in fatty infiltration of liver and ketosis[3,16,18,29]. It was shown that total bilirubin levels in group I ($p<0.01$) and direct bilirubin levels in clinical ketosis group ($p<0.001$) increased significantly. According to these results higher increase in indirect bilirubin compared with direct bilirubin in clinical ketosis group showed liver damage[28].

Serum albumin level decreases when there is hepatic lipidosis and severe liver damage[16,27,30,31]. In this study it was observed that albumin levels decreased ($p<0.05$). This is supported by West[18] who reported that estimation of albumin level is a good indicator of synthesis capacity of liver.

ß Hydroxybutyrate concentration was positively corraleted with GLDH, AST, indirect bilirubin ($p<0.001$) and total bilirubin ($p<0.01$) levels in clinical and subclinical ketosis groups. These results are in agreement with those of other studies[3,24,32,33] that show the significance of GLDH and AST in determining liver finctions in dairy cows.

In this study it was observed that estimation of serum ß Hydroxybutyrate concentration is of great value in diagnosis of subclinical ketosis, and also there is liver damage in cows with ketosis and evaluation of AST and especially GLDH levels is very important for diagnosis and determining the severity of liver damage.

As a result it was concluded that economic losses can be minimized by early diagnosis of ketosis by estimating serum ß Hydroxybutyrate concentrations periodically during the last month of gestation and within three months after calving in large farms with high yielding cows.

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