Investigation of the Prevalence of Ketosis in Cows in Ardahan Region

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ABSTRACT

In the postpartum period, the energy requirement increases in high yielding dairy cows. According to the negative energy balance (NED) degree, clinical or subclinical ketosis may occur during this period. In this study; The aim was to investigate the prevalence of ketosis in dairy cows in Ardahan Region. The animal material to be used in the study was determined as 200 as a result of statistical analyzes of TÜİK data. Animal material was selected from Ardahan city center, Göle, Çıldır, Hanak and Damal districts. The enterprises where, study is carried out are similar in terms of milk yield, management, maintenance and nutrition factors. Blood samples were collected on days 7 and 14 postpartum to determine the prevalence of ketosis. Beta hydroxybutyrate (BHB), non-esterified fatty acid (NEFA) and glucose levels were determined from the obtained samples. Patients with BHB concentration with 1.0<1.4 mmol / L were considered to have subclinical ketosis. Patients with BHB concentration with ≥1.4 mmol / L were considered to have clinical ketosis. The prevalence of clinical ketosis in the postpartum period in Ardahan was 1% (2/200) and the prevalence of subclinical ketosis in Ardahan was 10% (20/200). Blood glucose levels of animals with ketosis were significantly lower than the healthy animals.

Keywords: β-hydroxybutyrate, clinical ketosis, subclinical ketosis, non-esterified fatty acid, negative energy balance

Ardahan Yöresindeki İneklerde Ketoaz Yayılımının Araştırılması

ÖZ

Postpartum dönemde, yüksek verimli süt ineklerinde enerji gereksinimini artırmaktadır. Bu dönemde oluşabilen negatif enerji dengesinin (NED) derecesine göre, klinik veya subklinik kozotiz meydana gelebilmektedir. Bu çalışmada; Ardahan yöresinde süt ineklerindeki kozotiz yayılımının arastırılması amaçlanmıştır. Çalışmada kullanlacak hayvan materyali sayısını TÜİK verilerine göre yapılan istatiksel analizler sonucunda 200 olarak belirlendi. Hayvan materyali, Ardahan Merkez, Göle, Çıldır, Hanak ve Damal ilçelerinden seçildi. Çalışma yapılan işlemler; süt verimi, yönetim, bakım ve beslenme faktörlerini açısından birbirine benzer olarak seçildi. Kozotizin prevalansını belirlemek amacıyla ka numuneleri doğumdan sonra 7 ve 14. günlerde toplandı ve elde edilen örneklerden Beta Hidroksibütürat (BHB), NEFA ve glukoz seviyeleri belirlendi. BHB konsantrasyonu, 1.0-1.4 mmol/L arasında olanlar subklinik kozotizli olarak kabul edildi. BHB konsantrasyonu ≥1.4 mmol/L olanlar ise klinik kozotiz olarak kabul edildi. Çalışma sonucunda Ardahan yöresinde postpartum dönemde klinik kozotiz yayılımı %1 (2/200) ve subklinik kozotiz yayılımı ise %10 (20/200) olarak tespit edildi. Kozotizli hayvanların kan glukoz seviyesi sağlıklı hayvanlara göre önemli derecede düşük tespit edildi.

Anahtar Kelimeler: β-hydroxybutyrate, klinik kozotiz, subklinik kozotiz, esterleşmemiş yağ asidi, negatif enerji dengesi
**INTRODUCTION**

In dairy cows, the period including three weeks before and after birth is called the transition period. Metabolic changes occurring in this period are observed to be higher than those occurring during pregnancy and lactation (Grummer 1995). In particular, problems originating from metabolism lead to a significant yield decrease, and to reproductive losses (Drackley 1999, İssi et al. 2016). Any health problem that occurs in the cows in the transition period decreases milk yield by an average of 7.2 L daily during the first 20 days of lactation (Vernon 2005).

Infertility and metabolic diseases which are characterized by decreased milk yield and by yield losses, are the most important problems of the transition period (Issi et al. 2016). The decrease in dry matter intake (DMI) is the most important risk factor in the development of these diseases. Metabolic diseases are observed in the first weeks of lactation, where milk synthesis increases rapidly (Şahal et al. 2011, Yıldız et al. 2019). Ketosis develops in cows with high milk yield as a result of the disruption of carbohydrate and volatile fatty acid metabolism in the two-month postpartum period, especially in the 2nd to 4th weeks. The disease is characterized by decreased blood glucose level, depletion of liver glycogen and glucose reserves, decreased gluconeogenic activity, fatty degeneration in the liver and increased ketone bodies in the body. Ketosis is a disease of metabolism that has an acute, subacute and chronic course (Blood and Radostits 1989, Drackley et al. 1992, Yuhang et al. 2015, Hossain and Samad 2019). Ketone bodies (BHB, acetoacetic acid and acetone) are formed as a result of fatty acid oxidation. Low blood glucose level during ketosis triggers the mobilization of fat reserves in the body and thus the level of NEFA increases (Ospina et al. 2010). With this study, the prevalence of ketosis in the cows in the Ardahan region and the economic losses of this disease were investigated by examining the levels of BHB, NEFA, Glucose, Triglyceride, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Calcium (Ca) and Phosphorus (P) on the 7th and 14th days after birth.

**MATERIALS and METHODS**

**Animal material**

According to the data of Ardahan Agriculture Provincial Directorate, there are 131,942 dairy cows between the ages of 3-7 in Ardahan region, considering the number of animals from which blood and milk will be taken, it has been calculated that 196 dairy cows will be sufficient at %95 confidence interval. In this study, 200 cows belonging to a total of 97 breeders in the age range of 3-7 years were used.

**Ethical Approval**

This research has been approved by Kafkas University Animal Experiments Local Ethics Committee (KAU-HADYEK/2018-092).

**Collection of blood samples**

Cows were sampled on postpartum days 7 and 14. For examination, 5 mL of blood was taken from V. jugular for his sampling. Blood samples were taken 4-5 hours after feeding (Duffield, 2000; Öğün, 2008). Blood samples were taken into an antiagulant-free (BD Vacutainer, UK) and Sodium fluoride (NaF) tubes, and incubated at room temperature for 20 minutes and then centrifuged at 3000 rpm for 15 minutes (Custer et al., 1983). After serum samples were taken, glucose and BHB were determined. The sera were then stored at -20 °C until further analysis.

**BHB, NEFA, Glukoz, Trigliserit, AST, ALT, Ca and P measurements**

Serum BHB (Randox®, United Kingdom), NEFA (Randox®, United Kingdom), Glukoz (DDS®, Turkey), Trigliserit (DDS, Turkey), AST (DDS, Turkey), ALT (DDS, Turkey) Calcium (DDS, Türkiye) and Fosfor (DDS, Türkiye) were measured using commercial ELISA kits.

**Statistical analysis**

Data was analysed using statistical software package SPSS® (SPSS 22, USA). The comparison of two parameters was made using t test. One-way analysis of variance (ANOVA) was used to determine changes in parameters over time. The results obtained were expressed as mean and standard error (X±SE). Values of P<0.05 and below were considered statistically significant.

**RESULTS**

In the study, as stated in the method, those with BHB levels between 1.0-1.4 mmol/L were considered as subclinical, those with ≥1.4 mmol/L were considered as clinical ketosis and those with <1.0 mmol/L as healthy. According to this procedure, the prevalence of ketosis on the 7th day is presented in Table 1. On the 7th day of postpartum, subclinical ketosis prevalence in Ardahan region was found to be at the rate of 10% (20/200) and clinical ketosis prevalence was found to be at the rate of 1% (2/200) (Table 1).
Table 1: Prevalence of ketosis according to BHB level on postpartum 7th day
Tablo 1: Postpartum 7. gün BHB seviyesine göre ketozis prevalansı

|          | N  | Rate (%) | Min | Max. | Mean | SE  |
|----------|----|----------|-----|------|------|-----|
| Healthy  | 178| 89       | 0.62| 0.84 | 0.74 | 0.06|
| Subclinical ketosis | 20| 10       | 1.05| 1.39 | 1.23 | 0.10|
| Clinical ketosis  | 2 | 1        | 1.41| 1.91 | 1.66 | 0.35|

The prevalence of ketosis on postpartum 14th day is presented in Table 2. In this period, subclinical ketosis prevalence in Ardahan region was found to be at the rate of 8.5% (17/200) and clinical ketosis prevalence was found to be at the rate of 0.5% (1/200) (Table 2).

Table 2: Prevalence of ketosis according to BHB level on postpartum 14th day
Tablo 2: Postpartum 14. gün BHB seviyesine göre ketozis prevalansı

|          | N  | Rate (%) | Min | Max. | Mean | SE  |
|----------|----|----------|-----|------|------|-----|
| Healthy  | 182| 91       | 0.56| 0.96 | 0.65 | 0.06|
| Subclinical ketosis | 17| 8.5      | 1.02| 1.36 | 1.13 | 0.10|
| Clinical ketosis  | 1 | 0.5      | 1.78| 1.78 | 1.78 | 0.00|

In the study, comparison of some biochemical parameters in the postpartum 7th and 14th days in cows with and without ketosis (healthy) is shown as Mean ± SE in Table 3. On the postpartum 7th and 14th days, it was determined that NEFA, triglyceride, AST and ALT levels increased significantly (P <0.001), while glucose level decreased (P <0.001) compared to healthy cows. However, at that sampling time, there was no significant change encountered in Ca and P levels (Table 3).

Table 3: Comparison of some biochemical parameters in the postpartum 7th and 14th days in cows with and without ketosis
Tablo 3: Sağlıklı ve ketozisli ineklerin postpartum 7 ve 14. günlerde bazı biyokimyasal parametrelerinin karşılaştırılması

| Parameters           | Healthy     | Ketosis (Subclinical and Clinical) | P      |
|----------------------|-------------|-----------------------------------|--------|
| Postpartum 7th day   |             |                                   |        |
| NEFA (mmol/L)        | 0.42±0.01   | 0.81±0.01                         | P<0.001|
| Glukoz (mg/dL)       | 50.69±0.13  | 45.45±0.4                         | P<0.001|
| Trigliserid (mg/dL)  | 22.69±0.10  | 31.86±0.58                        | P<0.001|
| AST (U/L)            | 81.19±0.42  | 116.68±2.11                       | P<0.001|
| ALT (U/L)            | 26.03±0.22  | 36.68±1.05                        | P<0.001|
| P (mmol/L)           | 2.50±0.01   | 2.49±0.02                         | P>0.05 |
| Ca (mmol/L)          | 1.47±0.01   | 1.47±0.02                         | P>0.05 |
| Postpartum 14th day  |             |                                   |        |
| NEFA (mmol/L)        | 0.45±0.00   | 0.77±0.01                         | P<0.001|
| Glukoz (mg/dL)       | 55.33±0.11  | 46.05±0.33                        | P<0.001|
| Trigliserid (mg/dL)  | 19.85±0.11  | 29.95±0.50                        | P<0.001|
| AST (U/L)            | 78.33±0.35  | 109.37±2.27                       | P<0.001|
| ALT (U/L)            | 24.40±0.19  | 34.64±1.00                        | P<0.001|
| P (mmol/L)           | 2.50±0.01   | 2.50±0.19                         | P>0.05 |
| Ca (mmol/L)          | 1.48±0.01   | 1.49±0.02                         | P>0.05 |
According to the survey information obtained from the producers, daily milk yields in the cows with ketosis (subclinical and clinical) and in healthy cows in winter (from 1 November to 31 March) and pasture periods (from 1 April to 30 October) are presented in Table 4. In the performed analyses, it was determined that the milk yield decreased significantly (P < 0.01) in animals with ketosis (Table 4).

Table 4. Periodical daily milk yield in ketosis and healthy cows (L/day)

| Period          | Availability | N  | Mean | SE  | P       |
|-----------------|--------------|----|------|-----|---------|
| Pasture Period  | Healthy      | 75 | 11.14| 0.24| P<0.01  |
|                 | Ketosis      | 22 | 9.45 | 0.48|         |
|                 | Healthy      | 75 | 5.81 | 0.20| P<0.01  |
| Winter Period   | Ketosis      | 22 | 4.40 | 0.32|         |

DISCUSSION

Ketosis is a metabolism disease which is acute, subacute and has chronic course and characterized by disruption of carbohydrate and volatile fatty acid metabolism, depletion of glycogen and glucose reserves in the liver, fat degeneration and decreased glucose level due to these disorders, and increased ketone bodies (Blood and Radostits 1989). Symptoms of clinical ketosis are clinical symptoms and ketonuria in urine and milk. However, subclinical ketosis, which leads to secondary diseases (Mastitis, Metritis etc.) and progresses latently without showing clinical information, causes serious economic losses (Ögün 2008). This is usually because subclinical ketosis is not diagnosed and is overlooked.

It has been reported that almost half of the high-milk-producing cows, in particular, carry a risk of subclinical ketosis during the early lactation period (Emery et al. 1968, Ögün 2008). In a study conducted in the neighbouring city of Kars, where BHB was used as a criterion, the prevalence of subclinical ketosis was determined as 12.02% on the postpartum 7th day and 10.3% on the 14th day (Ögün 2008). In a study conducted in 12 countries in North America and Western Europe between 2011 and 2013, the prevalence of subclinical ketosis in the holstein breed has been determined as 24.1% (Brunner et al. 2019).

In a study conducted in the Mediterranean, Aegean and Marmara regions in the postpartum period, the clinical ketosis prevalence has been found as 3.8%, 7.3% and 9.7%, respectively. In the same study, the prevalence of subclinical ketosis has been found to be 14.8%, 16.6% and 22.3%, respectively (Şentürk et al. 2016). In our study, the clinical ketosis rate was determined as 1% and 0.5%, and the rate of subclinical ketosis as 10% and 8.5%, respectively, in postpartum 7th and 14th days. The determined rates, along with being close to those of Turkey, are seen to be lower than of those in Europe. This situation is thought to be closely related to nutritional programs and milk yield. It has been reported that the incidence of ketosis can be determined at the highest level in the first week of postpartum (Emery et al. 1968, Ögün 2008).

It was reported that the level of BHB decreased significantly in the postpartum 14th day compared to the 7th day (Cavestany et al. 2005, Ögün 2008). Also in this study, it was found that the level of BHB decreased similarly in different breeds and in total.

It has been reported that the level of NEFA and then BHB increase in ketosis (Veennon et al. 1991). Increased levels of BHB may be accompanied by increased NEFA and decreased glucose (Aeberhard et al. 2001, Busato 2002, Ögün 2008, Akgül 2014). In this presented study, it was determined that there was a decrease glucose level and an increase in NEFA and BHB in cows with ketosis (subclinical and clinical).

Table 4. Periodical daily milk yield in ketosis and healthy cows (L/day)

| Period          | Availability | N  | Mean | SE  | P       |
|-----------------|--------------|----|------|-----|---------|
| Pasture Period  | Healthy      | 75 | 11.14| 0.24| P<0.01  |
|                 | Ketosis      | 22 | 9.45 | 0.48|         |
|                 | Healthy      | 75 | 5.81 | 0.20| P<0.01  |
| Winter Period   | Ketosis      | 22 | 4.40 | 0.32|         |

In the transition period, especially during the lactation phase, when the energy used in the body tissues and milk production cannot be met with ration, the energy deficit is met by the mobilization of fats (Bertics et al. 1992, Ögün 2008). However, the amount of fatty acid that can enter the TCA cycle is limited. When this limit is exceeded, the level of NEFA increases (Goff and Horst 1997). In the presented study, it was found that the level of NEFA in cows with ketosis increased compared to the healthy ones. It has been reported that the NEFA level had increased in cows with NEB and this was associated with increased fat mobilization (Aeberhard et al. 2001).
It has been determined that the glucose level was the lowest at the postpartum 8th day and increased until the 21st day (Seifi et al. 2007). In addition, it has been reported that glucose level decreased by 25% in the first week of lactation compared to the prenatal period and increased in the second week of lactation (Vaquez-anon et al. 1994). The level of glucose and ketone bodies may provide information about the amount of energy required in animals (Herdt et al. 1981). Low glucose and high BHB levels also indicate that energy is not taken enough (Whitaker et al. 1983). It has been stated that glucose level is a good indicator in determining the severity of the disease in clinical ketosis (Kelly 1977). Decrease in glucose value has been determined to be parallel with the increase in the level of BHB (Andre et al. 1987). It has been reported that glucose level decreased dramatically in cows with ketosis in the postpartum period (Akgül 2014). In another study conducted in the same period, it has been determined that glucose level decreased significantly in cows with ketosis (El-deep and El-bahr 2017). In this presented study, it was determined that the cows with ketosis had a low glucose level in the postpartum 7th and 14th days compared to healthy ones. In addition, glucose level was determined to be increased significantly on the 14th day compared to the 7th day. This indicates that the glucose level decreases in the postpartum process and increases gradually after the first week. It has been reported that decreased glucose is associated with insufficient liver function, low energy in feed and increased glucose requirement (Aslan and Nizamiloğlu 1985, Duffield 2000, Veenhuisen et al. 1991, Öğün 2008). During the transition phase of dairy cows, the need for glucose increases for lactose production (Busato et al. 2002). This leads to the development of NEB and a decrease in glucose as a result of insufficient gluconeogenesis (Andersson and Emanuelson 1985, Brumby et al. 1975, Drackley 1999).

In this study, it was determined that the level of triglyceride increased on the postpartum 7th day and decreased on the 14th day. In the postpartum first two weeks in the cows with ketosis, triglyceride levels were found higher than healthy ones. It has been reported that circulating triglycerides are taken by the mammary glands in high-milk-yield cows in lactation period and used in milk fat synthesis (Grunmer 1993). NEFA is the main component for triglyceride (Akgül 2014). Goff and Horst (1997) have reported that triglyceride level varies depending on nutrition. It has been determined that in the postpartum first two weeks in cows with ketosis (subclinical and clinical), AST and ALT activities are considerably high compared to healthy ones. In addition, it has been determined that the level of AST and ALT was high on the postpartum 7th day and then decreased on the 14th day. AST activity has been reported to be increased on the postpartum 7th day and then decreased gradually (Seifi et al. 2007). In subclinical ketosis, AST and ALT activities have been found to be increased (Öğün 2008). Similarly, it has been reported that AST and ALT activity was increased in cows with subclinical ketosis, however, this increase was lower in ALT (Kenneman 1999). Studies and our study show that the increase in AST and ALT activity is associated with liver fattening and ketosis (Kauppinen 1984, Steen et al. 1997, El-deep and El-bahr 2017).

It has been reported that the level of Ca in cows varies between 2.3 mmol/L and the P level between 1.16-2.32 mmol/L (Barton et al. 1981, Can et al. 1987, Öğün 2008). The level of Ca and P has been reported to be decreased on the postpartum 8th day, then increased gradually, and this was due to the use of molecules in milk synthesis (Seifi et al. 2007). In this presented study, no changes in Ca and P values were detected in the postpartum 7th and 14th days in cows with ketosis and in healthy animals and in between these days. In a similar study, it has been reported that Ca level was lower in cows with ketosis than healthy ones, however, the difference was not significant (Akgül 2014).

CONCLUSION

As a result, it was determined that the prevalence of ketosis can be determined by the level of BHB. In the cows in Ardahan region, the clinical ketosis prevalence was determined on the postpartum 7th day as 1%, subclinical ketosis prevalence was determined as 10%, while the clinical ketosis prevalence was determined on the 14th day as 0.5% and subclinical ketosis prevalence was determined as 8.5%. According to the measurements performed on the postpartum 7th and 14th days with the BHB test, the most sensitive breed to clinical ketosis risk was detected to be Brown Swiss hybrid and the most sensitive breed to the risk of subclinical ketosis was detected to be Brown Swiss. In the cows with ketosis, BHB level decreases on the postpartum 7th day compared to 14th day. In our study, it was determined that milk yield decreased by 25% in ketosis disease. When this result was adapted to TSI data, it was found that a daily financial loss of 76,193.25 TL occurred in Ardahan and its vicinage.

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