

The effect of serum β -hydroxybutyric acid and calcium levels on left displaced abomasum in Holstein cows on transition period

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ABSTRACT

In this study, the effect of serum β -hydroxybutyric acid and calcium concentrations, which are the parameters used in the diagnosis of ketosis and hypocalcemia in lactation period, on left displaced abomasum (LDA) has been investigated. The lactation period covering the 3 weeks before and after parturition, known as the transition period, is highly important for high yield dairy cows (Holstein Friesian cattle). Hormonal and metabolic changes occur in this period. The energy requirement, which increases in direct proportion with the milk yield at the beginning of lactation, cannot be met with insufficient dry matter consumption, however, it is compensated with the mobilization of body fat. Ketosis and fatty liver are nutritional diseases that are observed in animals with high milk yield resulting from the disturbances in energy metabolism. Hypocalcemia and ketosis are the most important risk factors in the development of left displaced abomasum (LDA) in high-yield milk cows. Hypocalcemia and ketosis are also the most important nutritional diseases in the transition period. In this study, 17 Holstein Friesian cattle were used in group 1 that were diagnosed with left displaced abomasum after hearing the “ping” sound, and 17 healthy, randomly selected Holstein Friesian cattle were used in group 2. Blood analyses (BHBA and Ca) were performed in group 1 and 2 after parturition

Keywords: ketosis, left displaced abomasum, β -hydroxybutyric acid, calcium

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Introduction

Left displaced abomasum (LDA) is an economically significant nutritional disease that is common in dairy cows in gestation period. The lactation period covering the 3 weeks before and after parturition, known as the transition period, is highly important for high yield dairy cows (Holstein Friesian cattle). Hormonal and metabolic changes occur in this period. The energy requirement, which increases in direct proportion with the milk yield at the beginning of lactation, cannot be

met with insufficient dry matter consumption, however, it is compensated with the mobilization of body fat. Nutritional diseases observed in animals with high milk yield such as displaced abomasum, hypocalcemia, ketosis, fatty liver, mastitis, metritis, retained placenta, acidosis and laminitis are all associated with one another, and presentation of one of these causes the appearance of others.

Nutritional diseases observed in high yield dairy

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cows in transition period due to inaccurate care and nutrition are: Energy and mineral metabolism disorders and immune system disorders experienced due to the negative energy balance that occurs upon insufficient food and dry matter intake. The appearance of one of these nutritional diseases in high milk yield cows triggers another, and causes the presentation of diseases that are associated with each other. The incidence of left displaced abomasum is high in cows with hypocalcemia and ketosis. The most important factor in displaced abomasum is abomasal atony. Meanwhile, atony is caused by traumatic reticuloperitonitis, ulcer, metritis, mastitis, retained placenta, acidosis, increased volatile fatty acids and low blood calcium levels. In left displaced abomasum, uterus expands towards the end of pregnancy, gets under the rumen and lifts the rumen up. Abomasum enters the space previously occupied by rumen. Shrinking in size after parturition, the uterus retreats back to its own space, and rumen descends toward the space left by the uterus again, leaving the abomasum stuck in the place it entered before. As being stuck between the rumen and left abdominal wall, and the abomasum gets "displaced" to the left. The abomasum is displaced to the left in 75% of cases (Geishauser, 1995; Van Winden and Kuiper, 2003; Alaçam, 2011)

There is a positive correlation between non-esterified fatty acids, which took form after fatty acid mobilization, and negative energy level and nutritional diseases. Increases in non-esterified fatty acids in the blood, hormonal changes, increased requirement of nutrients (a pregnant cow needs 75% more nutrients than non-pregnant cow at the same weight), and increases in feed consumption are observed upon parturition (while a non-pregnant cow consumes dry matter at 2% of its live weight, a pregnant cow consumes 1.4% of its live weight in dry matter). Negative energy balance is observed in 80% of high yield milk cows. At negative energy balance, anestrus period is extended, corpus luteum functions are weakened, and cystic ovary and metritis cases are high (Alaçam, 2011; Overton and Waldron, 2004; Alaçam et al., 2008; Van Saun, 2004). Immune system is suppressed as a result of the excretion of minerals and fat-soluble vitamins with milk, and it results in immune dysfunction (Curtis et al., 1983).

Ketosis is a nutritional disease characterized with degeneration of the liver, decreased blood glucose concentration, increased level of ketone bodies in the blood and other body tissues (acetoacetic acid, β -hydroxybutyric acid and acetone) observed in postpartum period. As a result of body fat

mobilization in negative energy table, plasma concentration of free fatty acids is increased in various tissues. Free fatty acids in the liver are limited with the oxidation capacity of the liver. Upon exceeding this capacity, free fatty acids are converted into triglycerides, and accumulated in the liver. The prevalence of ketosis increases when plasma concentration of free fatty acids exceeds 1000 mEq/ L (Arslan and Tufan, 2010).

Depending on the energy deficit of the animal in negative energy level, increased blood levels of non-esterified fatty acids resulting from the mobilization of body fat storage are transported to the liver and undergo incomplete oxidation, resulting in the formation of ketone bodies that participate in milk formation in mammary glands, and thus the milk fat ratio is increased. Due to low levels of fermentable non-structural carbohydrates in the ration feed, inability of rumen bacteria to multiply, insufficient energy in rumen, insufficient microbial protein synthesis in rumen and decreased level of metabolized proteins in the small intestine causes insufficient amino acid level in mammary glands. This causes decreases in milk protein ratio (Hayırlı and Çolak, 2011).

In a non-pregnant cow, abomasum resides on the ventral wall in abdominal cavity, slightly to the right of the median line. With pregnancy, the space occupied by the uterus increases in the abdominal cavity. The uterus pushes rumen forward and the abomasum shifts to the left, and after parturition, abomasum goes back to its original place. The gases formed as a result of the fermentation of feed in the abomasum are sent back to the rumen. As a result of feeding the cow with a ration that includes higher level of concentrate feed compared to roughage, the level of gas formed in abomasum increases and the abomasum expands, decreasing its capacity to move. Expanded abomasum starts to spread out to the left, and gets between rumen and abdominal wall. Limited feed consumption causes decreased fullness in the rumen, and this poses a risk for displaced abomasum (Biricik, 2012).

In this study, the effect of ketosis and hypocalcemia on left displaced abomasum (LDA) was investigated.

Materials and Methods

Sample collection: In this study, there were 17 Holstein Friesian cattle in group 1 that were diagnosed with left displaced abomasum (LDA) in the auscultation performed between 11th-13th intercostal space in the first postpartum 30 days, by

hearing the “Ping” sound with tympanic resonance, and there were 17 Holstein Friesian cattle in group 2 that were healthy, randomly selected and gave birth within the last 30 days.

Biochemical analysis: Serum NEFA level is not used in our study since nonesterified fatty acids (NEFA) are esterified after parturition and converted into triglycerides (reesterified triglycerides), or ketone bodies as β -hydroxybutyric acid (BHBA). Serum BHBA level was designated as $BHBA \geq 1.200$ mmol/L in the diagnosis of ketosis, and total Ca level was designated as 1.875- 2.075 mmol/L in the diagnosis of hypocalcemia. Blood samples were taken from V. Coccylgea of the cows in group 1 that were diagnosed with displaced abomasum and the healthy cows in group 2 that gave birth in the last month, samples were taken to the laboratory by cold chain, separated from the serum was obtained by centrifugation at 3500 rpm for 5 min, and analyzed Serum Beta hydroxy butyric acid (BHBA) and total calcium (Ca) levels have been analyzed in Biotechnica Instruments BT 3500 device by using commercial kits.

Ration analysis: In this study, cows were fed with Total Mixed Ration (TMR). The contents of TMR were 25 kg of corn silage, 3 kg of wheat straw, 2 kg of hay and 9 kg of dairy cattle feed. The cows have consumed 39 kg of natural feed daily, 19.6 kg on dry matter basis. The ratio of roughage / concentrated feed in the ration was: 61.2/ 38.8. Nutrient composition of TMR is presented in Table 1 on dry matter basis.

Statistical analysis: Obtained results have been evaluated with SPSS V.21 package program. The distribution of results was controlled with Levene’s test, calcium level with t test, and BHBA levels were evaluated with Mann Whitney U test. Mean results of groups and standard errors are presented in Table-2. Box plots were established for visualizing the distribution of data. Statistical significance level was determined as $p < 0.001$. Serum BHBA and total Ca levels of the cows in group 1 that were diagnosed with displaced abomasum and the healthy cows in group 2 have been compared, and the differences between the groups were determined to be significant at $P < 0.001$ level for both features. The approval of local ethics committee dated 12.03.2018 nr. 2018/ 5 has been obtained from Etlik Veterinary Control Central Research Institute Ankara /Turkey.

Results

Definition of left displaced abomasum is “Ping” sound on abdominal percussion. The symptoms of ketosis are decrease in milk yield, reduced feed intake and decreased appetite/refusing concentrate intake, excessive loss of body condition, constipation, ketone

odor in breath /milk and nervous signs (weakness, mania, apparent blindness and pica). The cows in this study were fed with Total Mixed Ration (TMR), and nutrient composition of TMR is presented in Table 1 on dry matter basis. Statistical results of the cows in group 1 that were diagnosed with displaced abomasum and the healthy cows in group 2 that gave birth in the last month for serum BHBA and total Ca levels are also presented. As it is known, hypocalcemia was diagnosed within the first postpartum 3 days by checking the symptoms and serum total Ca level, and ketosis was diagnosed within the first postpartum week by checking the symptoms and serum BHBA level.

Table 1. Nutrient composition of TMR (DM %)

DM (dry matter) %	50.7
CP % (Crude protein)	12.8
CF % (Crude fat)	2.6
CC % (Crude cellulose)	20.6
NDF % (Neutral detergent fiber)	44.2
CA % (Crude ash)	6.6
NEL Mcal/kg (Net energy lactation)	1.39

Figure 1 shows the BHBA level in cows that were diagnosed with left displaced abomasum. BHBA level was determined to be higher in cows diagnosed with displaced abomasum compared to healthy cows. The difference between cows diagnosed with displaced abomasum and healthy cows was determined to be statistically significant with regard to BHBA level ($p < 0.001$). Calcium results are presented in Figure 2. Upon comparing mean results between the two groups, Ca level was determined to be higher in healthy cows compared to cows diagnosed with displaced abomasum. The difference between the Ca levels of cows diagnosed with left displaced abomasum and healthy cows was determined to be statistically significant ($p < 0.001$).

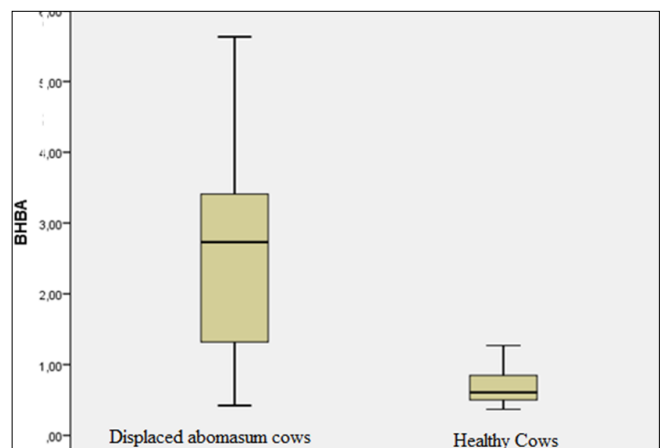


Figure 1. BHBA levels in cows diagnosed with left displaced abomasum and healthy cows

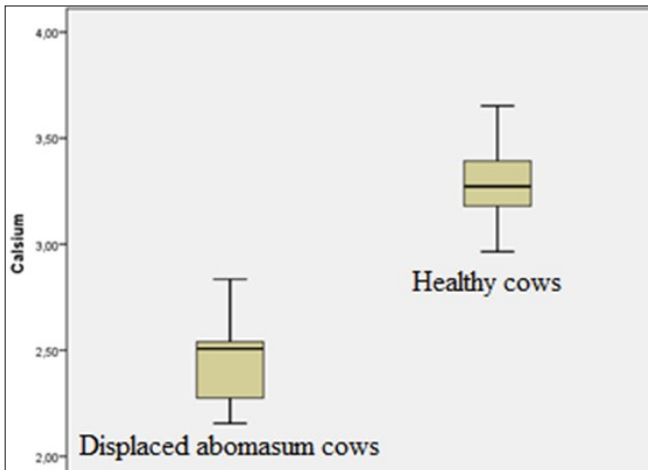


Figure 2. Calcium levels in cows diagnosed with left displaced abomasum and healthy cows.

Discussion

With the start of lactation, serum glucose and insulin levels are decreased in dairy cows while beta hydroxy butyric acid (BHBA) and non-esterified fatty acid (NEFA) levels increase (Van Winden et al., 2003).

It has been stated that the risk of displaced abomasum increases with the decreased consumption of roughage and dry matter in the last two weeks of dry period, slow increase of dry matter and concentrated feed consumption with parturition, and decreased fullness capacity of rumen as a result of decreased roughage/concentrated feed ratio. As a result of the decrease in roughage, and the increase in concentrated feed and volatile fatty acids upon parturition, decreased rumen motility reduces the absorption of volatile fatty acids. Gases accumulated in the rumen proceed into the abomasum and allows the abomasum to move. Also, there is a risk of LDA in cows with hypocalcemia upon parturition (Shaver, 1997).

The risk of displaced abomasum in cows with hypocalcemia is 4.8 times higher compared to those that do not have hypocalcemia. It has been indicated that LDA risk increases if total serum Ca level falls below 2.04 upon parturition (Massey et al., 1993).

Rumen activity is lost upon 2.2 mg/dl blood calcium level. Decreased rumen activity causes gas accumulation in rumen and abomasum, and decreases in abomasum activity (Jorgensen et al., 1998).

Subclinical BHBA level varies between 1.200- 1.400 mmol/L (Suthar et al., 2013). Clinical BHBA level is above 1.400 mmol/L. A high rate of blood BHBA concentrations has been determined in cows with displaced abomasum. BHBA levels have been found as 1.56 and 0.90 mmol/L, respectively, in cows with

displaced abomasum compared to control group (Stengarde et al., 2010). In our study, mean serum BHBA level was determined as 2.51 mmol / L in cows diagnosed with displaced abomasum.

BHBA concentration was increased on postpartum day 3 in cows diagnosed with displaced abomasum, however, Ca concentration was determined to be decreased (Hädrich, 2006). These results are determined to be in line with the results of our study.

In a study, BHBA level was determined as 1.29 ± 0.13 mmol/L for subclinical ketosis, and BHBA level was determined as 2.49 ± 0.17 mmol/L for clinical ketosis (Şentürk et al., 2016). It has been stated that hypokalemic abomasal displacement cows have important deviations in their energy and fat metabolism for acid-base and electrolyte status compared to normokalemic cows, and negative events are experienced in the course of disease for this reason. BHBA has been stated to cause a negative effect on abomasal displacement DA cows (Alexandra, 2014).

It was indicated that increased BHBA concentration and decreased Ca concentration in the herd after parturition was associated with increased DA, and DA risk was elevated as a result. It was indicated that 1.400 mmol/L higher BHBA level and 2.1 mmol/L lower Ca level in the herd after parturition increased the possibility of displaced abomasum in the herd, and that 800 mmol/L lower BHBA level and 2.1 mmol/L lower Ca level was associated with decreased milk yield (Chapinal et al., 2012).

In a study comparing left displaced abomasum (LDA) cows and healthy cows, a statistical difference was observed between serum BHBA levels after parturition ($p=0.001$) (Cardoso et al., 2008). These results are determined to be in line with the results of our study.

In the study performed by Sarashina et al. (1989), the gas in the abomasum has originated from the rumen. While ruminal CO₂/CH₄ gas ratio is 2.01 in healthy cows, CO₂/CH₄ gas ratio is 0.44 in abomasum. Ruminal CO₂/CH₄ gas ratio dropped to 1.62 in displaced abomasum, and abomasal CO₂/CH₄ ratio has dropped to 0.35. CO₂ has been absorbed from the abomasal wall, and therefore CO₂/CH₄ gas ratio in the abomasum has changed. Unlike the gas in the rumen, abomasal CH₄ has been found to be higher than CO₂ gas both in normal and DA cows (Sarashina, 1990).

In general, 1.70 mmol/L serum Ca concentration has been reported to cause decreased feed consumption due to the decrease in ruminal activity

(Batmaz, 2015), and Ca concentration below 1.2 mmol/L has been reported to cause decreased abomasal motility (Madison and Troutt, 1998). This value was suggested to be associated with displaced abomasum as a result of decreased abomasal motility.

Eight times higher displaced abomasum prevalence has been reported in cows that had serum BHBA levels of > 1.200 mmol/L after parturition. Elevation of serum BHBA level after parturition has been significantly associated with the subsequent increase in LDA risk. Displaced abomasum is a more important risk factor than ketosis and hypocalcemia. Prepartum nonesterified fatty acid (NEFA) value both shows and reflects the severity of negative energy balance, and plays a key role in LDA etiology (Leblanc et al., 2005).

In general, feed consumption and milk yield drops in cows with DA, and serum Ca level is decreased while BHBA level is increased (Van Winden et al., 2003).

It has been stated that DA risk is 13.6 times higher in cows with ≥ 1000 mmol/L serum BHBA level, compared to cows with lower serum BHBA levels. It has also been indicated that ketosis risk is 4.7 times higher in cows with ≥ 1200 mmol/L serum BHBA level. It has been determined that 2.2 mmol/L lower serum total Ca concentration after parturition increases the risk of displaced abomasum (Seifi et al., 2011). These

results are determined to be in line with the results of our study.

Postpartum BHBA concentration at critical threshold value of 1.000 mmol/L should be assessed as a risk factor for displaced abomasum (Ospina et al., 2010).

Conclusion

Consequently, as a reflection of the negative energy balance after parturition, high BHBA level causes the presentation of ketosis. Reducing nutritional diseases bears vital importance for the health and yield of the herd. It has been concluded that subclinical and clinical hypocalcemia reference levels should be re-interpreted due to the high yield of dairy cows and increased dry matter consumption. There is a strong relation between the manifestation of ketosis after parturition and the presentation of displaced abomasum in the first postpartum month. Ketosis is observed as a result of the negative energy balance presented due to dry matter consumption and decreased appetite. It is considered that left displaced abomasum is inevitable after the diagnosis of ketosis with the increased ruminal fullness and decreased abomasum motility. Furthermore, feeding cows with negative ration cation-anion balance in dry period will decrease the risk of hypocalcemia.

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