

Reproductive Performance, Milk Yield Parameters, and Some Postpartum Diseases That Are Associated with Clinical And Subclinical Ketosis in Dairy Cows

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ABSTRACT

Negative energy balance (NEB) is a physiological state that arises during the transition from pregnancy to lactation in dairy cows. This transition period is associated with a decline in dry matter intake and an increased demand for energy sources for milk production. Ketosis, a metabolic disorder, often emerges shortly after calving and is characterized by reduced glucose levels, depleted liver glycogen, and elevated ketone body concentrations. The study aimed to investigate the impacts of ketosis on milk yield, reproductive parameters, and postpartum metabolic diseases in cows with subclinical and clinical ketosis. The study involved 267 healthy pregnant Holstein dairy cows in the second and third lactation periods. Groups were formed based on postpartum third-day beta-hydroxybutyric acid (BHBA) levels. The groups included control (BHBA < 1.2 mmol/dL; n=101), subclinical ketosis (BHBA = 1.2-2.4 mmol/dL; n=90), and clinical ketosis (BHBA > 2.4 mmol/dL; n=76) groups. Results indicated that higher body condition scores (BCS) during calving were associated with an increased risk of postpartum ketosis (p<0.001). Subclinical ketosis led to a higher number of inseminations per pregnancy (p<0.01), extended service periods (p<0.001), and decreased pregnancy rates at first insemination (p>0.05). It was observed that the clinical ketosis group had a longer time to reach peak milk yield (p<0.001) and a higher peak milk yield (p<0.01). The incidence of septic metritis was found to be higher in cows with clinical ketosis (p<0.01). Consequently, the study revealed that ketosis has negative effects on reproductive parameters and the incidence of metabolic diseases. Additionally, the importance of BCS in assessing and managing the risk of ketosis in dairy cows during the transition period was emphasized. It is believed that further evaluation of the study with more animal material and additional parameters could yield more meaningful results.

Key Words: cattle, feeding, mastitis, metritis negative energy balance

Sütçü Sığırlarda Klinik ve Subklinik Ketozis ile İlişkili Üreme Performansı, Süt Verim Parametreleri ve Bazı Postpartum Hastalıklar

ÖZ

Negatif enerji dengesi (NED), süt sığırlarında gebelikten laktasyona geçiş sırasında ortaya çıkan fizyolojik bir durumdur. Bu geçiş dönemi, kuru madde alımındaki azalma ve süt üretimi için artan enerji ihtiyacı ile ilişkilidir. Ketozis, sıklıkla doğumdan hemen sonra ortaya çıkan bir metabolik bozukluktur ve düşük glukoz seviyeleri, azalmış karaciğer glikojeni ve artan keton cisimcikleri konsantrasyonları ile karakterizedir. Bu çalışma, subklinik ve klinik ketozise sahip ineklerde ketozisin süt verimine, üreme parametrelerine ve postpartum metabolik hastalıklara etkilerini araştırmayı amaçlamıştır. Çalışma, ikinci ve üçüncü laktasyon dönemlerindeki 267 sağlıklı, gebe Holstein ineklerini kapsamaktadır. Gruplar, doğum sonrası üçüncü gün beta-hidroksiyaütirik asit (BHBA) seviyelerine göre oluşturuldu. Gruplar, kontrol (BHBA < 1.2 mmol/dL; n=101), subklinik ketozis (BHBA = 1.2-2.4 mmol/dL; n=90) ve klinik ketozis (BHBA > 2.4 mmol/dL; n=76) gruplarından oluşmaktadır. Sonuçlar, doğum sırasında daha yüksek vücut kondisyon skorlarının (VKS) doğum sonrası ketozis riski ile ilişkilendirildiğini göstermiştir (p<0.001). Subklinik ketozis, gebelik başına daha fazla tohumlama (p<0.01), servis periyodunun uzaması (p<0.001) ve ilk tohumlama gebelik oranlarında düşüşe (p>0.05) yol açmıştır. Klinik ketozis grubunun pik süt verimine ulaşma süresinin daha uzun olduğu (p<0.001) ve daha yüksek pik süt verimine (p<0.01) sahip olduğu görüldü. Klinik ketozis olan ineklerde septik metritis insidansının (p<0.01) daha yüksek olduğu tespit edildi. Sonuç olarak, çalışmada ketozisin, üreme parametreleri ve metabolik hastalık insidansı üzerinde olumsuz etkilere sahip olduğunu görüldü. Ayrıca, süt sığırlarının geçiş döneminde ketozis riskini değerlendirmede ve yönetmede VKS'nin önemi vurgulandı. Çalışmanın daha fazla hayvan materyali ve daha fazla parametre ile değerlendirmesinin daha anlamlı sonuçlar verebileceği düşünülmektedir.

Anahtar Kelimeler: sığır, besleme, mastitis, metritis, negatif enerji dengesi

To cite this article: Cambaz AÖ, Bozkurt G. Reproductive Performance, Milk Yield Parameters, and Some Postpartum Diseases That Are Associated with Clinical And Subclinical Ketosis in Dairy Cows. Kocatepe Vet J. (2023):16(4):588-596

Submission: 14.09.2023 Accepted: 11.12.2023 Published Online: 14.12.2023

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INTRODUCTION

Negative energy balance (NEB) is a physiological condition that occurs during the transition from pregnancy to lactation in dairy cattle. Dry matter consumption decreases by more than 30% in the last 3 weeks of pregnancy (Hayirli et al., 2002). The transition from late pregnancy to early lactation is a risky period for dairy cattle, during which many infectious and metabolic diseases can occur (Compton et al., 2014; Ingvarthsen et al., 2003). During this period, the demand for energy sources such as glucose, amino acids, and fatty acids for milk production is 2-5 times higher than the pre-calving period requirements (Bell, 1995). Cows are in NEB when the energy from the diet cannot provide enough energy for high milk production (Grummer, 1995).

Ketosis is a metabolic disease in cattle that often begins in the first few days after birth and seriously affects animals for up to 2 weeks (Suthar et al., 2013). NEB is characterized by decreased glucose levels, reduced liver glycogen stores, fatty degeneration in the liver, and an increased concentration of ketone bodies in the blood, urine, milk, and respiratory air (Bremmer, 2002). Ruminants are susceptible to ketosis, and this susceptibility can be exacerbated in cows due to their increased glucose requirement (Drackley et al., 2001). From the start of the lactation period, until the cow reaches peak milk yield, there is a physiological loss in the cow's body condition. This loss is accompanied by an increase in beta-hydroxybutyric acid (BHBA) levels (Le Blanc, 2010).

Ketosis is classified in different ways and the one of classification is related to BHBA concentrations. It is classified as subclinical and clinical ketosis, depending on the level of BHBA in the blood and the presence or absence of clinical signs of disease. Subclinical ketosis is defined as an increase in ketone bodies in the blood. The obvious finding of the disease is the presence of ketone bodies in the blood, urine, or milk. The serum BHBA values (1200-1400 $\mu\text{mol/L}$) are generally used for the diagnosis of subclinical ketosis (Suthar et al., 2013). Clinical ketosis is characterized by the presence of clinical symptoms such as hyperketonemia, hypoglycemia and decreased appetite, weight loss, and decreased milk yield. Clinical ketosis is usually determined at higher BHBA concentrations in the blood (Gordon et al., 2013).

This study aimed to determine the potential adverse effects of disease-related ketosis on some milk yield and reproductive parameters and the incidence of postpartum metabolic diseases in cows with subclinical and clinical ketosis.

MATERIALS AND METHODS

Animal Material and Feeding Management

The study includes a total of 267 pregnant Holstein dairy cows in their second and third lactation periods. The animals had an average weight of 746 ± 71 kg, and their average daily milk yields ranged from 38 to 40 L. Throughout the lactation period, the cows' milk yield was recorded as 11,000-12,000 kg. The animals are grouped based on the number of milking days, reproductive statuses, and milk yields. The cows are milked three times a day. The animals included in the study were fed two different rations during the dry period. These rations were the far-off dry period ration consumed from 39-60 days before calving and the close-up dry period ration consumed before calving. The animals had ad libitum feeding. The compositions of the late dry and close dry period rations are presented in Table 1. Following calving, two different rations were used during the early lactation (days 1-21) and mid-late lactation (days 22-305) periods (Table 2).

Study Groups

The study consisted of three different groups; control (BHBA < 1.2 mmol/dL; n=101), subclinical ketosis (BHBA = 1.2-2.4 mmol/dL; n=90), and clinical ketosis (BHBA > 2.4 mmol/dL; n=76). The body temperatures of the animals included in the study groups were monitored for the first 3 days after calving. During this period, cows with diseases such as mastitis or retained placenta were not included in the groups. All the animals in the study were in second and third lactation periods.

Determination of BHBA Levels and Body Condition Scores

Blood samples were collected from the jugular vein from the all animals. Blood BHBA levels were measured on postpartum days 3, 7, and 21. In the formation of groups, postpartum 3rd day BHBA levels were taken as criteria (Suthar et al., 2013). The measurements of BHBA levels were conducted using Centrivet GK blood ketone strips rapid test kit (mmol/dL Catalog No: 1150872301). The determination of body condition scores (BCS) in the study groups was done considering the same days (55 days before calving – dry period and 73 days after calving – first insemination time). The method of Wildman et al. (1982) was used as a reference for BCS determination.

Treatment

No treatment was administered to cows in the subclinical ketosis group. However, cows showing clinical ketosis (BHBA > 2.4 mmol/dL) based on the BHBA result obtained on the 7th day were recorded and excluded from the study, and treatment was applied. Cows in the clinical ketosis group were

subjected to a ketosis treatment protocol. For this purpose, intravenous administration of 500-1000 cc of 30% dextrose (DekstroVIP®, % 30, Polifarma) and glucocorticoid (Devamed®, Topkim) were applied (Gordon et al., 2013). Animals that did not recover and had mixed infections were removed from the groups and transferred to the routine management treatment protocol.

Synchronization Protocol and Pregnancy Examination

A voluntary waiting period of 73 days was determined on the farm. During this period, no artificial insemination was applied to the animals. The synchronization protocol started on postpartum days 53-55. The double-ovsynch synchronization protocol was applied. For synchronization, 0.075 mg of cloprostenol (Dalmazin®, Fatro-Güneşli A.Ş.) and 0.0262 mg/ml of Lecirelin (Dalmarelin®, Fatro-Güneşli A.Ş.) were used. The first pregnancy examination for all study groups was conducted through ultrasonography (Hasvet 838, Türkiye) on days 34±3 after insemination. The second pregnancy examination was performed on days 63±3 of pregnancy.

Reproductive and Milk Yield Parameters

The number of inseminations per pregnancy, service period, and conception rate in the first insemination were recorded in the herd management program (Delpro™, Unided Kingdom). The 305-day milk yield, time to reach peak milk yield and peak milk yield of cows in the study group were recorded. Data collection was carried out through a parallel milking system (DeLaval) during milking and obtained based on three milkings per day from the program.

Detection of Postpartum Diseases

Postpartum routine controls were conducted every morning during milking (mammary and milk examination). During the controls, body temperature, fecal examination, and lochia discharge were evaluated. Additionally, feed consumption and rumination were observed. Suspected animals were brought to the clinic for treatments and diagnoses were confirmed. Left displaced abomasum (LDA) was determined by abdominal ping sound with a stethoscope (Niehaus, 2008). Septic metritis was characterized by vaginal examination revealing dark-colored, malodorous discharge ranging from dark brown to reddish, containing small fetal membranes (Azawi, 2008). Hypocalcemia was diagnosed through clinical examination (Quader et al., 2017). Similarly, animals diagnosed with mastitis through clinical examination, mammary examination, and somatic cell count (California mastitis test) were recorded (Ruegg and Reinemann., 2002; Sargeant et al., 2001).

Statistical Analyses

SPSS 25.0 software package was used for statistical analysis of the data in the study. A mixed model was constructed to examine the effect of ketosis on milk and reproductive parameters, considering the impact of body condition scores. The Bonferroni test was applied to assess the significance of the difference between groups. The chi-square test was used to determine the difference in the incidence rates of metabolic diseases among study groups. Statistical significance was accepted as $p < 0.05$. Results were presented as mean ± standard error.

Ethics Approval

All procedures were approved by the Animal Ethics Committee (AEC) at Burdur Mehmet Akif University, Turkiye (No: 113/1147)

RESULTS

BHBA Levels and Body Condition Score

Mean BHBA levels were determined as 0.84 ± 0.07 mmol/L in the control group, 1.47 ± 0.09 mmol/L in the subclinical ketosis group, and 3.15 ± 0.54 mmol/L in the clinical ketosis group on the postpartum 3rd day. Seventh and 21st day blood BHBA levels are shown in Table 3.

It was observed that the control group had a BCS of 3.43 ± 0.06 , while the subclinical and clinical ketosis groups had BCS values of 3.57 ± 0.07 and 3.69 ± 0.07 , respectively ($p < 0.001$). It was determined that the clinical ketosis group had the highest BCS during the dry period. The changes in BCS during the dry period and at the time of first insemination among the study groups are shown in Table 4.

Some Reproductive Parameters

The number of inseminations per pregnancy was determined as 2.47 ± 1.22 for the control group, 3.43 ± 1.22 for the subclinical ketosis group, and 2.99 ± 1.23 for the clinical ketosis group. A statistically significant difference was found among the groups, with the subclinical ketosis group having a higher number of inseminations per pregnancy compared to the other groups ($p < 0.01$). When evaluated in terms of the service period, the values for the control, subclinical, and ketosis groups were determined as 124.77 ± 7.56 , 160.51 ± 8.01 , and 176.76 ± 9.35 days, respectively. A shortened service period was observed in the control group ($p < 0.001$). In the study, it was determined that 39.6% of the cows in the control group, and 27.78% and 37.88% of the cows in the subclinical and clinical ketosis groups, respectively, became pregnant at the first insemination (Table 5). There was no statistically significant difference among the groups in terms of pregnancy rate at the first insemination ($p > 0.05$).

Milk Yield Parameters

The time to reach peak milk yield was found to be 60.04 ± 2.66 days for the control group, 67.88 ± 2.76

days for the subclinical ketosis group, and 82.41 ± 3.09 days for the clinical ketosis group. There was a statistically significant difference among the groups, indicating that healthy cows reached peak milk yield more quickly, while cows in the ketosis group took longer to reach it ($p < 0.001$). In terms of peak milk yield, the averages for the control, subclinical, and clinical ketosis groups were determined as 57.44 ± 1.77 , 58.17 ± 1.78 , and 60.79 ± 1.83 liters, respectively. The difference among the groups was statistically significant, with the highest peak milk yield observed in the clinical ketosis group ($p < 0.01$). There was no statistically significant difference among the study groups in terms of the 305-day milk yield averages ($p > 0.05$). Milk yield parameters based on groups are presented in Table 6.

The Rates of Some Diseases

The rates of septic metritis in the control, subclinical ketosis, and clinical ketosis groups were found to be 5.94%, 11.11%, and 25.76%, respectively. It was observed that the rates of septic metritis were higher in cows with clinical ketosis ($p < 0.01$). The incidence of hypocalcemia was 0.99% in the control group, 1.11% in the subclinical ketosis group, and 4.55% in the clinical ketosis group. There was no significant difference in the frequency of hypocalcemia among the study groups ($p > 0.05$). The rates of LDA were 2.97% in the control group, 10% in the subclinical ketosis group, and 13.64% in the clinical ketosis group. It was determined that the rate of LDA was lower in the control group compared to the other groups ($p < 0.05$). The proportions of cows with septic metritis, clinical hypocalcemia, mastitis, and LDA based on the study groups are shown in Table 7.

Table 1. The ration of dry period

Ration	Far of Dry Period (kg/cow)	Close up Dry Period (kg/cow)
Alfalfa Hay	1.00	0
Wheat Straw	8.00	5.00
Corn Silage	13.00	8.00
Grass Silage	3.75	4.75
Canola Meal	1.90	1.80
Premix	0.01	0.02
Zeolite	0.02	0.02
Urea	0.07	0
Marble Dust	0.25	0.32
Magnesium Oxide	0.04	0.03
Dicalcium Phosphate (DCP)	0.10	0
Ammonium Chloride	0	0.25
High Moisture Corn	0	3.90
Orange Pulp	0	2.00
Calculated Nutrient Values	Values	Values
Crude Protein, %	11.57	12.90
Dry Matter Intake, kg/day	15.72	12.75
Roughage Ratio in Diet, %	88.66	100
Concentrate Ratio in Diet, %	11.34	0
Soluble Protein, %	5.67	7.83
Soluble Crude Fiber, %	9.25	4.43
Neutral Detergent Fiber (NDF), %	53.58	44.90
Non-Fiber Carbohydrates (NFC), %	25.81	31.23
Sugar, %	3.02	2.50
Starch, %	9.83	20.53
Calcium (Ca), %	1.19	0.99
Phosphorus (P), %	0.30	0.32

Magnesium (Mg), %	0.51	0.42
Sodium (Na), %	0.09	0.07
Chloride (Cl), %	0.54	1.53
Dietary Cation-Anion Difference (DCAD), mEq/100g	16.8	19.50

Table 2. The ration of lactation period

Ration	Early Lactation (kg/cow)	Mid and late lactation (kg/cow)
Alfalfa Hay	5.00	4.60
Cottonseed	1.45	1.50
Cracked Corn	1.00	1.15
Soybean Meal	0.75	1.00
Corn Gluten Meal	0.60	0.85
Sodium Bicarbonate	0.35	0.40
Bypass Fat	0.45	0.35
Canola Meal	1.25	1.70
Premix	0.35	0.35
Marble Dust	0.30	-
High-Moisture Corn	3.50	7.00
Corn Silage	13.25	29.50
Corn Distillers Dried Grains with Solubles (DDGS)	-	0.80
Potato Pulp	-	2.00
Orange Pulp	-	4.00
Water	3.50	4.00
Molasses	0.60	0.50
Calculated Nutrient Values	Values	Values
Dry Matter Intake kg/day	17.89	27.03
Crude Protein %	17.35	16.25
Soluble Protein %	8.40	7.60
NDF (Neutral Detergent Fiber) %	29.49	27.53
ADF (Acid Detergent Fiber) %	21.21	18.6
Sugar %	5.33	4.50
Starch %	21.25	27.75
NFC (Non-Fiber Carbohydrate) %	38.96	45.02
Soluble Fiber %	8.98	8.74
Fat %	5.57	4.92
NEL (Net Energy for Lactation), Mcal/kg	1.97	1.66
Calcium %	1.07	0.48
Phosphorus %	0.39	0.36
Magnesium %	0.52	0.38
Sodium %	0.71	0.52
Chlorine %	0.47	0.37
DCAD (Dietary Cation-Anion Difference) mEq/100g	41.75	26.03

Table 3. BHBA levels according to days (mmol/L)

	Control	Subclinical ketosis	Clinical ketosis
3 rd day	0.84±0.07	1.47±0.09	3.15±0.54
7 th day	0.82±0.01	1.01±0.25	1.27±0.14
21 st day	0.85±0.05	0.88±0.04	0.96±0.09

Table 4. Body condition scores according to groups

	Control	Subclinical ketosis	Clinical ketosis	<i>P value</i>
Dry period	3.43±0.06 ^c	3.57±0.07 ^b	3.69±0.07 ^a	***
First insemination	2.90±0.06	2.87±0.06	2.85±0.06	-
Difference	0.53±0.07 ^c	0.71±0.08 ^b	0.84±0.08 ^a	***

a, b, c: There is a statistically significant difference between different letters on the same row.

***: (p<0.001).

Table 5. Some reproductive parameters

	Control	Subclinical ketosis	Clinical ketosis	<i>P value</i>
NOIP	2.47±1.22 ^b	3.43±1.22 ^a	2.99±1.23 ^{ab}	**
SP (day)	124.77±7.56 ^b	160.51±8.01 ^a	176.76±9.35 ^a	***
FSCR (%)	39.60	27.78	37.88	-

NOIP: Number of inseminations per pregnancy SP: Service period FSCR: First-service conception rate

a, b, c: There is a statistically significant difference between different letters on the same row

** : p<0,01; *** p<0,001

Table 6. Some milk yield parameters

	Control	Subclinical ketosis	Clinical ketosis	<i>P value</i>
TPMY (day)	60.04±2.66 ^c	67.88±2.76 ^b	82.41±3.09 ^a	***
PMY (Liter)	57.44±1.77 ^b	58.17±1.78 ^b	60.79±1.83 ^a	**
LMY (Liter)	12772.27±602.6	13104.76±605.53	12686.36±616.12	-

TPMY: Time to peak milk yield PMY: Peak milk yield LMY: Lactation milk yield

a, b, c: There is a statistically significant difference between different letters on the same row

** : p<0,01; *** p<0,001

Table 7. Incidence of some postpartum diseases according to groups (%)

	Control	Subclinical ketosis	Clinical ketosis	<i>P</i> value
Septic metritis	5.94 ^b	11.11 ^b	25.76 ^a	**
Clinical Hypocalcemia	0.90	1.11	4.55	-
Mastitis (10-30 days)	3.55 ^b	4.86 ^{ab}	6.14 ^a	*
LDA	2.97 ^b	10.00 ^a	13.64 ^a	*

LDA: Left displaced abomasum

a, b, c: There is a statistically significant difference between different letters on the same row

*: $p < 0,05$; **: $p < 0,01$; ***: $p < 0,001$

DISCUSSION

Ketosis is one of the most important metabolic diseases with a high prevalence in dairy cattle. Its prevalence was evaluated between DIM 2-21 days in studies conducted in different countries and different incidences were determined for subclinical ketosis and clinical ketosis (Lei and Simoes., 2021). Suthar et al. (2013) observed that the average prevalence of subclinical ketosis (BHBA ≥ 1.2 mmol/L) within ten European countries was 21.8% (ranging between 11.2 and 36.6%) between the 2nd and 15th day of lactation. In this respect, cattle with ketosis should be rapidly identified and treated.

Ketosis is directly related to BCS. Gillund et al. (2001) investigated the likelihood of ketosis between cows with a BCS ≥ 3.5 or higher and cows with a BCS score of 3.25 or lower during calving on 732 dairy cows. According to the obtained results, cows with a BCS ≥ 3.5 during calving were approximately 2.5 times more likely to develop ketosis compared to cows with a BCS score of 3.25 or lower. Additionally, cows experiencing significant BCS losses in the postpartum period were determined to have only half the probability of conceiving at the first insemination compared to cows with moderate BCS losses. Schrijver (2018) indicated that cows with high body condition scores during the dry period have a higher risk of elevated BHBA concentrations and developing ketosis in the postpartum period. Cows entering the dry period with high BCS were found to experience greater body condition losses in the first 8 weeks. Singh and Bhakat (2022) found in their study that overfed cows during the dry period became excessively conditioned at calving and experienced more severe NEB during the transition period. The presented study findings are consistent with previous reports. The current study demonstrates that cows with high BCS during calving are at a higher risk of postpartum ketosis. Cows with high body condition scores, when faced with increased energy requirements postpartum, start utilizing their energy reserves. This situation can contribute to the

development of ketosis by causing energy imbalance and accumulation of ketone bodies. Furthermore, the fact that ketosis groups are the ones with more severe BCS loss in the study supports this statement.

Ketosis results from the occurrence of an energy deficit due to severe NEB, negatively affecting certain reproductive performance parameters. Numerous studies have shown a relationship between reproductive traits, including the time from NEB to first luteal activity, the first conception interval, the time from calving to first ovulation, and the time from calving to conception (Gillund et al., 2001; Walsh et al., 2007). Duffield (2000) stated that cows with subclinical ketosis performed more insemination per pregnancy. Suthar et al. (2013) reported that BHBA concentrations exceeding thresholds of 1.1 and 1.6 mmol/L during the first 2 weeks postpartum reduced pregnancy probability. Additionally, it was found that the number of inseminations per pregnancy increased ketosis in Holstein cows with ketosis (Mellado et al., 2018). Raboisson et al. (2014) reported that both clinical and subclinical ketosis extended the first estrus interval and led to decreased pregnancy rates. In the current study, a statistically significant difference was observed in terms of number of inseminations per pregnancy between the control group and the subclinical ketosis group ($p < 0.001$). Conversely, such a difference was not found between the clinical ketosis and control groups. However, when evaluated proportionally, similarly to other studies, the number of inseminations per pregnancy increased by approximately 29% and 13% in the subclinical and clinical ketosis groups, respectively. The higher number of inseminations per pregnancy in the subclinical ketosis group may be attributed to the direct treatment of animals with the clinical form. Allowing animals in the subclinical ketosis group to undergo their physiological process may have an effect in this regard. Furthermore, as consistent with other studies, the pregnancy rate at first insemination decreased and the service period ($P < 0.001$) extended in these animals. The extended service period in the current study may be due to the

disruption of the initial ovulation process at the start of lactation due to increased NEB in cows. Another reason for the longer service period duration on the farm compared to the reviewed literature could be the higher average milk yield on this farm (Gorelik et al., 2021; Sehested et al., 2009)

The relationship between milk yield and ketosis generally varies according to the lactation period and parity (Ospina et al., 2010). Duffield et al. (2009) reported in their study on 1010 dairy cows that cows with subclinical ketosis during the postpartum period experienced a daily milk yield loss of 1.88 kg, whereas cows with clinical ketosis had a loss of 3.3 kg. Ospina et al. (2010) reported a reduction of 358 kg in the 305-day milk yield. McArt et al. (2012) stated that cows with a BHBA value of 1.2 mmol/L experienced a 0.5 L reduction in daily milk yield for every 0.1 mmol/L increase in BHBA on the 30th day postpartum. In this study, multiparous cows in their 2nd and 3rd lactation were used, and no differences were found among the groups in terms of the 305-day milk yield. The generally higher milk yield in cows with ketosis could contribute to greater increases in peak milk yield. The increase in milk yield at the beginning of the postpartum period and subsequently in peak yield might lead to higher fat mobilization and hence an increased risk of ketosis. Furthermore, since ketosis generally decreases after a maximum of 2 weeks, it can be among the reasons that the peak milk yield of high-yielding cows might not be significantly affected once peak production is achieved. Additionally, based on the results of this study, the extension of peak day duration due to NEB might lead to an increased peak milk yield in cows with ketosis.

Various studies indicate a relationship between ketosis and various diseases. The findings from these studies suggest that ketosis may be associated with LDA, metritis, and other diseases, which can have negative effects on reproductive efficiency (LeBlanc et al., 2005; Suthar et al., 2013; Seifi et al., 2011). Ospina et al. (2010), observed that there was a 1.8% increase in the incidence of LDA in animals with BHBA levels ≥ 1.2 mg/dL. Furthermore, LeBlanc et al. (2005), it was seen that cows with serum BHBA levels ≥ 1200 $\mu\text{mol/L}$ had a higher risk of LDA. These findings indicate a relationship between ketosis and the development of LDA. Cows with BHBA levels exceeding 1.2 to 1.4 mmol/L during the first 2 weeks postpartum were found to have higher likelihood of developing LDA compared to the control group (Suthar et al., 2013). Seifi et al. (2011) reported a higher risk of LDA in cows with ketosis in a weekly follow-up of 849 cows from 16 farms after calving. Similar results were obtained in the current study. Suthar et al. (2013) also noted in a study that subclinical ketosis was associated with metritis. Ospina et al. (2010) found an increased incidence of metritis in animals with BHBA levels ≥ 1.2 mg/dL. Similar results were obtained in the current study.

Ketosis increases the risk of septic metritis. In cows with ketosis, the increase in NEB levels can lead to immune deficiencies due to lymphocyte dysfunction (Schulz et al., 2015), which in turn can contribute to increased disease incidences.

CONCLUSION

In conclusion, it has been determined that both subclinical and clinical ketosis can lead to reproductive issues, potential problems in milk yield parameters, and associations with other metabolic diseases. This situation may also contribute to an increased frequency of metabolic disorders. This study has concluded that subclinical and clinical ketosis can lead to various negative effects. Furthermore, it emphasizes the importance of body condition score as a significant parameter in assessing and managing the risk of ketosis.

Conflict of Interest: The authors have no conflicts of interest to report.

Authors' Contributions: AOC and GB contributed to the project idea, design and execution of the study. All authors have write, read, and approved the finalized manuscript.

Ethical Approval: This research was approved by Animal Ethics Committee (AEC) at Burdur Mehmet Akif University (Ref No: 113/1147)

Acknowledgement: This study was summarised by the first author's high graduate thesis.

Explanation: None.

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