수의학석사 학위논문

Receiver Operating Characteristic Analysis for Prediction of Postpartum Metabolic Diseases in Dairy Cows in an Organic Farm in Republic of Korea

한국의 유기농 목장에서 분만 후 대사성 질병의 예측을 위한 ROC 분석

2023년 2월

서울대학교 대학원

수의과대학 산업동물임상의학 전공

김 도 희

Master’s Thesis of Veterinary Medicine

Receiver Operating Characteristic Analysis for Prediction of Postpartum Metabolic Diseases in Dairy Cows in an Organic Farm in Republic of Korea

한국의 유기농 목장에서 분만 후 대사성 질병의 예측을 위한 ROC 분석

February 2023

Department of Veterinary Medicine

Graduate School

Seoul National University

Major in Farm Animal Medicine

Dohee Kim

Receiver Operating Characteristic Analysis for Prediction of Postpartum Metabolic Diseases in Dairy Cows in an Organic Farm in Republic of Korea

Supervised by Professor Danil Kim

Submitting a master's thesis of Veterinary Medicine

October 2022

Department of Veterinary Medicine
Graduate School
Seoul National University
Major in Farm Animal Medicine

Dohee Kim

Confirming the master's thesis written by
Dohee Kim
January 2023

Chair ______________________(Seal)

Vice Chair ______________________(Seal)

Examiner ______________________(Seal)

Abstract

Postpartum diseases should be predicted to prevent productivity loss before calving especially in organic dairy farms. This study was aimed to investigate the incidence of postpartum metabolic diseases in an organic dairy farm in Korea, to confirm the association between diseases and prepartum blood biochemical parameters, and to evaluate the accuracy of these parameters with a receiver operating characteristic (ROC) analysis for identifying vulnerable cows.

Data were collected from 58 Holstein cows (16 primiparous and 42 multiparous) out of 94 Holstein parturient cows (25 primiparous and 69 multiparous) having calved for 2 years on an organic farm. During a transition period from 4 weeks prepartum to 4 weeks postpartum, blood biochemistry was performed through blood collection every 2 weeks with a physical examination.

Fourty-six cows among the 94 cows (48.9%) were diagnosed with at least one postpartum disease. Each incidence was 23.4% for subclinical ketosis, 19.1% for subclinical hypocalcemia, 13.8% for retained placenta, 10.6% for displaced abomasum and 6.4% for clinical ketosis.

Data from 58 Holstein parturient cows (16 primiparous and 42 multiparous) were divided into two groups: 31 cows with at least one disease and 27 cows without disease. Between at least one disease and no disease, there were significant differences in the prepartum levels of parameters like body condition score (BCS), non-esterified fatty acid (NEFA), total bilirubin (T-bil), direct bilirubin (D-bil) and NEFA to total cholesterol (T-chol) ratio ($p < 0.05$). The ROC analysis of each of these prepartum parameters had the area under the curve (AUC) < 0.7. However, the ROC analysis with logistic regression including all these parameters revealed a higher AUC (0.769), sensitivity (71.0%), and specificity (77.8%).

In summary, the ROC analysis with logistic regression including the prepartum BCS, NEFA, T-bil, D-bil, and NEFA to T-chol ratio can be used to identify cows that are vulnerable to postpartum diseases with a moderate accuracy.

Keyword : organic dairy farm, periparturient disease, metabolic parameters, ROC analysis, logistic regression
Student Number : 2020-22928

Contents

List of Tables

List of Figures

Literature review

Diseases caused by parturition in dairy cows are important because they are directly related to farm profitability. The period from 4 weeks before calving to 4 weeks after calving is called the transition period [Caixeta *et al*. 2021, Ospina *et al*. 2010]. Most transition cows enter a negative energy balance (NEB) state with reduced energy intake due to a decrease in dry matter intake (DMI) prior to calving, whereas energy requirements from milk production increase [Caixeta *et al*. 2021, Ospina *et al*. 2010]. When a cow's feed intake is insufficient to meet its energy requirements, hypoglycemia occurs, due to insufficient production of propionic acid, the main precursor of glucose in ruminants [Herdt *et al*. 2000]. Due to hypoglycemia, transition cows use adipose tissue for energy, which is mobilized as non-esterified fatty acids (NEFA) [Ospina *et al*. 2010, Herdt *et al*. 2000]. As the NEB condition persists, NEFA produced in excess interfere with hepatic metabolism and produce large amounts of ketone bodies, leading to ketosis. Beta-hydroxybutyrate (βHB) is synthesized from butyrate absorbed in the rumen epithelium and from hepatocyte ketogenesis from conversion of long-chain fatty acids due to fat mobilization [Asl *et al*. 1995]. The gold standard diagnostic test for subclinical ketosis is the measurement of stable βHB in serum [Asl *et al*. 1995, Herdt *et al*. 2000].

Elevated NEFA concentrations also cause triglyceride (TG) accumulation in the cytoplasm of hepatocytes, which is associated with a potential decrease in liver function [McSherry *et al*. 1984, Mostafavi *et al*. 2013, Ospina *et al*. 2010]. As DMI is reduced, production of very low-density lipoprotein (VLDL) is limited by low total cholesterol levels, and TG in the liver cannot be combined with VLDL and exported out of the liver, resulting in hepatic lipidosis (fatty liver) due to accumulation of fat [Kaneene *et al.* 1997, McSherry *et al*. 1984, Reinhardt *et al*. 2011]. Therefore, high NEFA to total cholesterol (T-chol) ratio values may suggest hepatic lipidosis [Kaneene *et al.* 1997, McSherry *et al*. 1984, Puppel *et al*. 2006]. In fact, one study identified major changes in the liver of fasted cows. Briefly, the volume density of cytoplasm occupied by glycogen decreased and the volume density of lysosomes and lipids increased, and the number of mitochondria per cell declined as the average mitochondrial volume was amplified [Holtenius *et al*. 1990, Reinhardt *et al*. 2011]. These changes in liver structure were accompanied by elevated serum enzyme activities (aspartate aminotransferase or sorbitol dehydrogenase) and escalated serum bilirubin concentrations [Mcsherry et al. 1984, Puppel *et al*. 2006, Reinhardt *et al*. 2011].

If cows do not obtain enough calcium from their bones and diet during the transition period to replace calcium lost to colostrum and milk production, they fail to maintain calcium homeostasis and may develop hypocalcemia [Caixeta *et al*. 2021, Chamberlin *et al*. 2013, Goff *et al.* 2008]. Low blood calcium levels lead to diminished ruminal and intestinal motility, and increased susceptibility to other metabolic and infectious diseases. In other words, hypocalcemia escalates the risk of displaced abomasum by reducing rumen and abomasal motility [Chamberlin *et al*. 2013, Goff *et al.* 2008]. Also, in expelling the placenta at calving, the risk of retained placenta may intensify due to difficulty in excretion because of reduced motility [Chamberlin *et al*. 2013, Goff *et al.* 2008].

Monitoring of cows during this transition is essential for early detection of disease signs, clinical and subclinical diagnoses, and overall management to prevent productivity losses [Caixeta *et al*. 2021]. To this end, several papers have emphasized the metabolic profile to predict disease before calving [Puppel *et al*. 2006]. The term 'metabolic profile' refers to the analysis of blood biochemical parameters that are useful for assessment and prevention of metabolic and nutritional disorders in dairy herds [Puppel *et al*. 2006, Van Saun *et al*. 2006].

In several studies, receiver operator characteristic (ROC) analysis has determined the threshold for serum concentrations of metabolic profiles associated with prediction of postpartum disease [Greiner *et al.* 2000]. In two studies, the critical threshold for prepartum NEFA to predict the development of displaced abomasum, clinical ketosis and retained placenta was 0.29 mEq/L and > 0.4mmol/L, respectively. [LeBlanc *et al*. 2010, Ospina *et al.* 2010) Another study established the cutoff points for prediction of subclinical ketosis as > 0.26 mmol/L for NEFA and < 2.26 mmol/L for glucose. [Asl *et al*. 1995]. Cows with NEFA concentrations ≥ 0.5 mEq/L between days 0 and 6 before calving were 3.6 times more likely to develop LDA after calving [LeBlanc *et al.* 2005].

In the case of βHB, prepartum βHB concentrations of ≥ 0.8 mmol/L were associated with postpartum diseases [Chapinal *et al*. 2011]. For calcium, cows with total respective blood calcium concentrations of ≤ 2.3 and ≤ 2.2 mmol/L, were 5 and 3 times more likely to develop displaced abomasum if threshold values were exceeded within the first postpartum week [Seifi *et al*. 2011, Chapinal *et al.* 2011]. Prepartum or postpartum cows with a serum total calcium of less than 8.0 mg/dl were 4 times more likely to develop postpartum disease problems [Van Saun *et al*. 2006]. Some researchers have suggested evaluating the NEFA to T-chol ratio. The critical threshold for prepartum NEFA to T-chol ratio to predict postpartum diseases was 0.2 [Van Saun *et al*. 2006].

Another study evaluated the increased odds association between the concentration of metabolic profile parameters and postpartum diseases [Quiroz-Rocha *et al*. 2009]. Low postpartum cholesterol in cows was associated with having one or more clinical diseases after calving, and for every 0.4 mmol/L decrease in serum cholesterol, cows were almost twice as likely to be diagnosed with multiple clinical diseases after calving [Sepulveda-Varas *et al.* 2015].

High serum concentrations of cholesterol and fatty acids were associated with increased odds of retained placenta. In fact, with each 0.1 mmol/L escalation in cholesterol or fatty acid concentrations in the week before parturition is associated with a 5% relative increase in the odds of retained placenta [Quiroz-Rocha *et al*. 2009]. Research on predicting postpartum diseases using metabolic profiles has continued.

Introduction

The transition period from 4 weeks before and after calving is crucial period for dairy cow [Grummer *et al.* 1995]. Because of the increased energy requirements for milk production and decreased DMI, most transition cows experience a NEB [Bell *et al.* 1995]. The NEB status induces lipid mobilization. Further, adipose mobilization is accompanied by high serum concentrations of NEFA [Herdt *et al.* 2000, Reid *et al.* 1977]. NEFA is transported for ketone body synthesis in the liver and is released into the blood to increase the concentration of ketone body [Grummer *et al.* 1993, McArt *et al.* 2012, Puppel *et al.* 2016]. Also, a sudden increase in calcium (Ca) demand at the onset of lactation can lead to hypocalcemia as calcium homeostatic mechanism does not adapt to the change in demand optimally [Goff *et al.* 2008]. These nutritional and physiological changes during the transition period increase the risk of metabolic and infectious diseases after calving in dairy cows [Ro *et al.* 2017].

Periparturient disease has a direct impact on the profitability of a dairy farm, owing to productivity loss as well as treatment costs [Caixeta et al. 2021]. Therefore, early prediction of the occurrence of periparturient disease and the execution of preventive measures through timely monitoring and intervention can reduce economic losses [Ro *et al.* 2017]. A metabolic profile refers to the analysis of blood biochemical parameters that are useful for the assessment and prevention of metabolic diseases in dairy cows [Puppel *et al*. 2016]. Using metabolic parameters, many studies have predicted postpartum diseases such as displaced abomasum [LeBlanc *et al*. 2010, Ospina *et al*. 2010], retained placenta [Kaneene *et al.* 1997, Ospina *et al*. 2010, Quiroz-Rocha *et al*. 2009], ketosis [Asl *et al*. 2011, Kaneene *et al.* 1997, Ro *et al.* 2017], and hypocalcemia [Chamberlin *et al*. 2013]. These parameters include NEFA, total cholesterol (T-chol), and β-hydroxybutyrate (βHB). Therefore, a method for predicting the occurrence of postpartum metabolic diseases by evaluating metabolic profiles is warranted.

Recently, with an increase in popularity of eco-friendly foods, interest in organic milk has grown as well. Organic milk refers to the milk that is produced in a farm that is certified as an organic product from the National Agricultural Products Quality Management Service in Republic of Korea. To obtain organic farm certification, organic feed and supplements are consumed by the cattle. Feed materials of organic farm may have narrower types and specifications of feed formulations, raw materials of feedstuff, and supplements compared to those of conventional farms. For example, with the restriction of fat protection-oriented organic supplementary feed that can supplement insufficient energy due to peak of milk yields in the early stage of lactation, farmer set the goal of milk yield lower than conventional farm and set feed mixing ratio based on nutritional balance. Moreover, various regulations are followed, such as prohibiting the use of antibiotics as well as other drugs. Due to a limited treatment regimen, parturient cows in organic farms might not receive active supportive care for adaptation to abrupt physiological changes as compared to cows in conventional farms. Therefore, it is important to predict the occurrence of postpartum diseases prior to parturition and to prepare preventive measures, especially for organic farms.

The objectives of this study were to investigate the incidence of postpartum metabolic diseases on an organic dairy farm in Korea and to confirm the association between the occurrence of metabolic diseases and prepartum blood biochemical parameters. Finally, the availability of prepartum blood biochemical parameters was evaluated to identify cows that are vulnerable to postpartum diseases, using a ROC analysis.

Material and Methods

1. Ethics

The owner knew and consented that blood tests and physical examinations during routine medical examinations be continuously recorded and that these contents be used in the laboratory process.

2. Experimental animals

In this study, data from 58 Holstein parturient cows (16 primiparous and 42 multiparous) out of 94 Holstein parturient cows (25 primiparous and 69 multiparous) were collected from November 2019 to November 2021. The data were collected in an organic farm located in Pyeongchang, Gangwon-do. Every 2 weeks (from 4 weeks prepartum to 4 weeks postpartum) periparturient cows were physically examined and their body condition score was determined by two experienced veterinarians [Ferguson *et al*. 1994]. Also, blood biochemical analysis was performed. The average milk production recorded by the robotic system (DeLaval, Sweden) was 24.9 L. The robot milking machine (automatic milking system) was allowed milking from 1.5 to 3 times a day on average. The cows were fed as shown in Table 1. Although organic farms may have a narrow range of feed formulations and specifications, efforts have been made to feed total mixed ration (TMR) to meet the nutritional requirements of each period (pre- and postpartum) of cows according to NRC (2001).

Table 1. Ingredient and nutrient composition of the prepartum and postpartum cow diet

Composition	Dry cow diet	Lactating cow diet
Ingredient (%)		
Formulated feed mixture	26.2	45.2
Silage	-	16.14
Alfalfa	-	10.90
Oat hay	36.9	8.72
Meadow hay	36.9	17.43
Kapok	-	1.61
Nutrient (%)		
DM (%)	88.0	78.61
CP (% of DM)	10.46	15.54
EE (% of DM)	1.92	3.64
CF (% of DM)	24.29	20.08
Ash (% of DM)	7.35	7.93
Ca (% of DM)	0.52	0.92
P (% of DM)	0.31	0.39
ADF (% of DM)	31.25	21.59
NDF (% of DM)	48.50	35.31
NFE (% of DM)	44.54	31.42
DM Intake (kg/day)	12-13	21.5

DM, dry matter; CP, crude protein; EE, ether extract; CF, crude fiber; ADF, acid detergent fiber; NDF, neutral detergent fiber; NFE, nitrogen free extract

3. Blood sampling

Samples of heparinized blood were collected from the coccygeal vessel. Samples in an ice box were sent to a laboratory. Plasma was separated and used for metabolic profile analyses, which included measuring the levels of total protein (TP), albumin (Alb), TG, T-chol, βHB, NEFA, glucose (Glu), total bilirubin (T-bil), direct bilirubin (D-bil), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), Ca, and inorganic phosphate (iP). An automatic analyzer BS-400 (Mindray, Shenzhen, China) was used. The data were divided into four time periods: 15-30 days before calving (BC-2), 1-14 days before calving (BC-1), 0-14 days after calving (AC+1), and 15-30 days after calving (AC+2).

4. Case definition

Postpartum metabolic diseases were investigated in a total of 94 Holstein parturient cows (25 primiparous and 69 multiparous) over a period of 2 years and based on the occurrence from calving to 30 days in milk. Retained placenta was defined as the failure to excrete the placenta by 24 hours after calving [LeBlanc *et al.* 2005, Ospina *et al*. 2010, Quiroz-Rocha *et al*. 2009]. Subclinical hypocalcemia was characterized as the plasma concentration of total Ca < 2.0 mmol/L [Reinhardt *et al.* 2011]. Cows were considered to have subclinical ketosis when the βHB concentration was 1.2 to 2.9 mmol/L [McArt *et al*. 2012], and clinical ketosis if the concentration was ≥ 3.0 mmol/L [Ospina *et al*. 2010]. Displaced abomasum was diagnosed by a veterinarian, based on the auscultation of a characteristic tympanic resonance during percussion [LeBlanc *et al.* 2005]. Subsequently, the incidence of postpartum metabolic disease was calculated. Appropriate veterinary treatment was provided for each disease immediately after a disease was diagnosed. Disease incidence in a total of 94 Holstein parturient cows, disease incidence in primiparous, and disease incidence in multiparous were also checked.

5. Grouping

For statistical analysis, cows were divided into two groups: cows with at least one disease and cows with no disease. Further statistical analysis was performed with 1) cows with subclinical disease vs. clinical disease vs. no disease 2) cows with one disease vs multiple diseases. Subclinical hypocalcemia and subclinical ketosis were included in the subclinical disease, and retained placenta, displaced abomasum, and clinical ketosis were included in the clinical disease.

6. Statistical analysis

Data were expressed as mean ± standard deviation (SD). According to the normality test results, parametric data were analyzed with Student's *t*-test, and non-parametric data were analyzed with Mann-Whitney U test to compare the significant differences in the parameters between diseased and non-diseased cows. To determine the cut-off values for predicting the occurrence of postpartum metabolic diseases, ROC analysis was performed on blood parameters from the two periods before calving. The point on the ROC curve with the highest combined sensitivity and specificity was regarded as the critical threshold [Greiner *et al.* 2000]. Interpretation of this cut-off point was based on the area under the curve (AUC) such that if AUC = 0.5 it was noninformative; if 0.5 < AUC ≤ 0.7, it was less accurate; if 0.7 < AUC ≤ 0.9, it was moderately accurate; if 0.9 < AUC < 1, it was highly accurate; and if AUC = 1, it was considered perfect [Greiner *et al.* 2000].

With all the parameters showing significant differences, the predicted probability model was obtained using logistic regression analysis. The predicted model is defined by following equation [Hosmer *et al*. 2000] :

$$P = \frac{1}{1+e^{-z}}$$

where, P is the predicted probability of periparturient disease occurrence, z is the linear combination, and the equation is [Hosmer *et al*. 2000]:

$$z = \beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\ldots\beta_nX_n$$

where β_0 is the intercept, the β_1, β_2, β_3 … β_n are the coefficients of logistic regression model, and X_1, X_2, X_3 … X_n are the independent variables. ROC curves were calculated to determine the sensitivity, specificity, and AUC of predictive models using logistic regression and presence or absence of postpartum disease.

Additionally, significant differences with parameters were also examined for diseased and non-diseased cows using only multiparous cows or primiparous cows. And, the significant difference for the two postpartum periods (AC+1 and AC+2) between diseased and non-diseased cows was compared with milk yield using Student's *t*-test. The BCS and blood parameters for subclinical vs. clinical vs. no disease cows were analyzed using one-way ANOVA followed by Tukey’s test at $p < 0.05$. Also, the parameters for one disease vs multiple diseases cows were analyzed using Student's *t*-test or Mann-Whitney U test. All statistical analyses were performed using SPSS version 25.0 (IBM SPSS Inc., Chicago, USA) and Table 2 summarizes the statistical analysis performed.

Table 2. A summary of the statistical analysis performed

Independent variable	Dependent variable	Comparison target	Statistical method
At least one disease and no disease cows	BCS, TP, Alb, TG, T-chol, βHB , NEFA, Glu, T-bil, D-bil, AST, GGT, Ca, iP, NEFA to T-chol ratio (metabolic profile analyses)	Total 58 periparturient cows	Student's *t*-test or Mann-Whitney U
At least one disease and no disease cows	BCS, NEFA, T-bil, D-bil, NEFA to T-chol ratio	Total 58 periparturient cows	ROC analysis
At least one disease and no disease cows	BCS, T-bil, D-bil, NEFA to T-chol ratio	Total 58 periparturient cows	Logistic regression & ROC analysis
At least one disease and no disease cows	BCS, metabolic profile analyses	16 primiparous or 42 multiparous	Student's *t*-test or Mann-Whitney U
At least one disease and no disease cows	Milk yield	Total 58 periparturient cows	Student's *t*-test
Subclinical, clinical and no disease cows	BCS, metabolic profile analyses	Total 58 periparturient cows	one-way ANOVA
One disease and multiple diseases cows	BCS, metabolic profile analyses	At least one disease cows	Student's *t*-test or Mann-Whitney U

BCS, body condition score; TP, total protein; Alb, albumin; T-chol, total cholesterol; βHB, β-Hydroxybutyrate; NEFA, non-esterified fatty acids; Glu, glucose; T-bil, total bilirubin; D-bil, direct bilirubin; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; Ca, calcium; iP, inorganic phosphate

Results

1. Incidence of postpartum diseases

A total of 46 parturient cows among the 94 cows (48.9%) were diagnosed with at least one disease after calving. The incidence of disease of 94 Holstein parturient cows was 22 cows with subclinical ketosis (23.4%), 18 with subclinical hypocalcemia (19.1%), 13 with retained placenta (13.8%), 10 with displaced abomasum (10.6%) and six with clinical ketosis (6.4%). The incidence of disease of 25 primiparous cows was six with subclinical ketosis (24%), five with subclinical hypocalcemia (20%), five with displaced abomasum (20%), three with retained placenta (12%) and two with clinical ketosis (8%). The incidence of disease of 69 multiparous cows was 16 with subclinical ketosis (23%), 13 with subclinical hypocalcemia (18.8%), 10 with retained placenta (14.5%), five with displaced abomasum (7.2%) and 4 with clinical ketosis (5.8%) (Table 3).

Table 3. The incidence of postpartum metabolic disease of 94 Holstein parturient cows, 25 primiparous cows and 69 multiparous cows

Postpartum metabolic diseases	Total (%)	Primiparous (%)	Multiparous (%)
Subclinical ketosis	22 (23.4%)	6 (24%)	16 (23%)
Subclinical hypocalcemia	18 (19.1%)	5 (20%)	13 (18.8%)
Retained placenta	13 (13.8%)	3 (12%)	10 (14.5%)
Displaced abomasum	10 (10.6%)	5 (20%)	5 (7.2%)
Clincal ketosis	6 (6.4%)	2 (8%)	4 (5.8%)
Total population	94	25	69

2. Statistical analysis

2-1. Significant difference in blood biochemistry parameters between diseased and non-diseased cows

In this study, parameters data from 58 Holstein parturient cows (16 primiparous and 42 multiparous) out of 94 Holstein parturient cows were collected. Fifty-eight cows were divided into two groups: 31 cows with at least one disease and 27 cows without disease.

At BC-2, there was no significant difference in blood biochemistry parameters between diseased and non-diseased cows except for BCS (p = 0.033) in Fig 1 - Fig 15. At BC-1, plasma concentrations of NEFA, T-bil, D-bil, and the NEFA to T-chol ratio were greater for cows in the periparturient disease group than those for cows in the non-periparturient disease group (p = 0.011, p = 0.017, p = 0.026, and p = 0.010, respectively). Interestingly, the aforementioned parameters of BC-1 were also significantly different between the groups at AC-1 and AC-2 ($p < 0.01$).

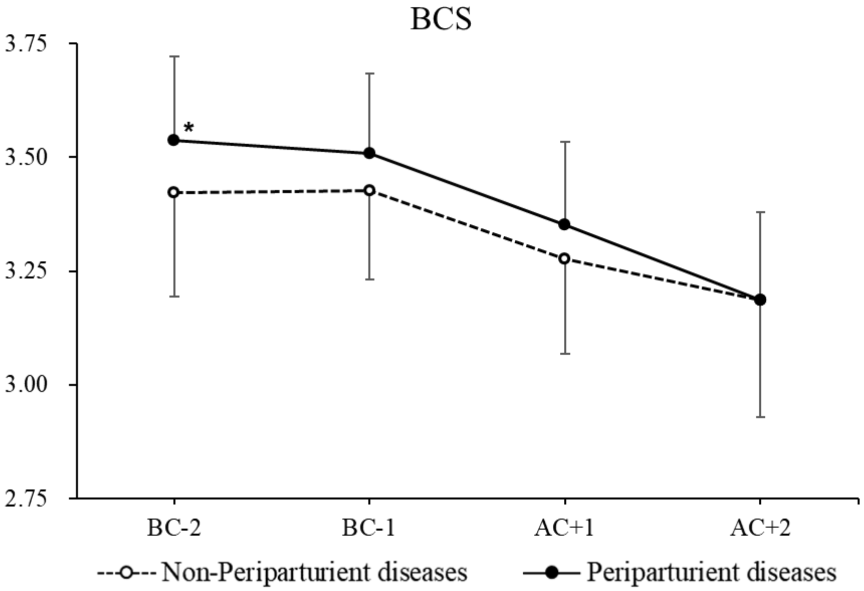

Fig. 1. Comparison of body condition score (BCS) in cows with periparturient disease and non-periparturient disease at for four time periods. Data are expressed as means ± SD. Significant difference between differences between periparturient disease cows and non-periparturient disease cows is indicated by *(p < 0.05) and **(p < 0.01).

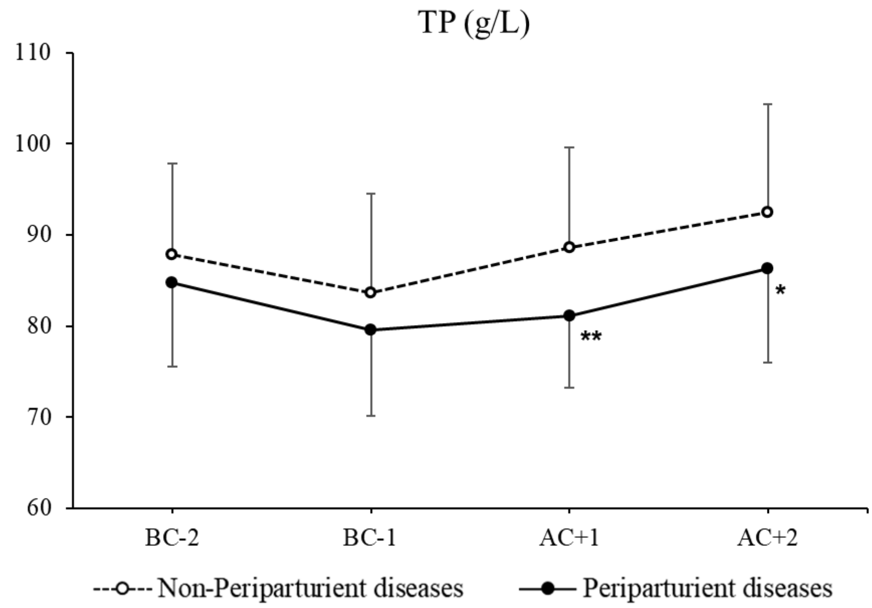

Fig. 2. Comparison of plasma total protein (TP) in cows with periparturient disease and non-periparturient disease at for four time periods. Data are expressed as means ± SD. Significant difference between differences between periparturient disease cows and non-periparturient disease cows is indicated by *(p < 0.05) and **(p < 0.01).

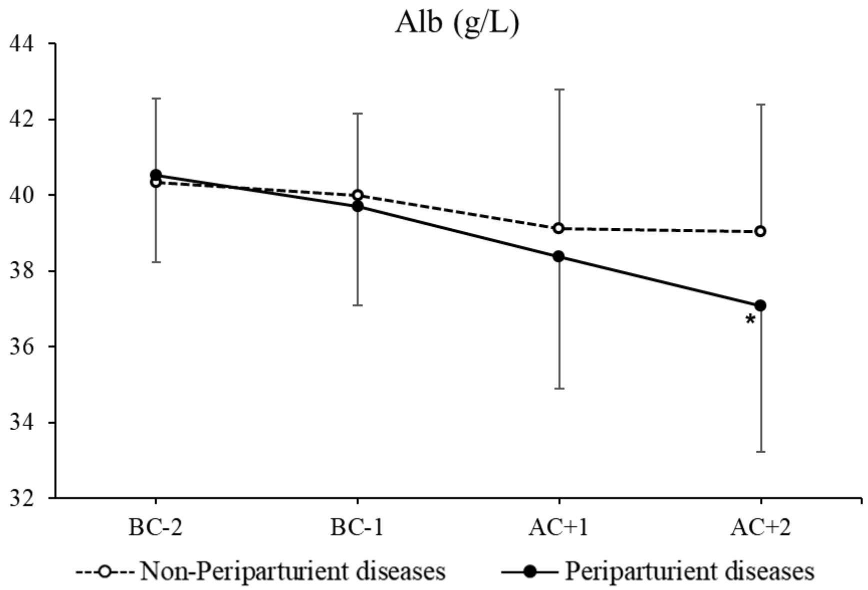

Fig. 3. Comparison of plasma albumin (Alb) in cows with periparturient disease and non-periparturient disease at for four time periods. Data are expressed as means ± SD. Significant difference between differences between periparturient disease cows and non-periparturient disease cows is indicated by *(p < 0.05) and **(p < 0.01).

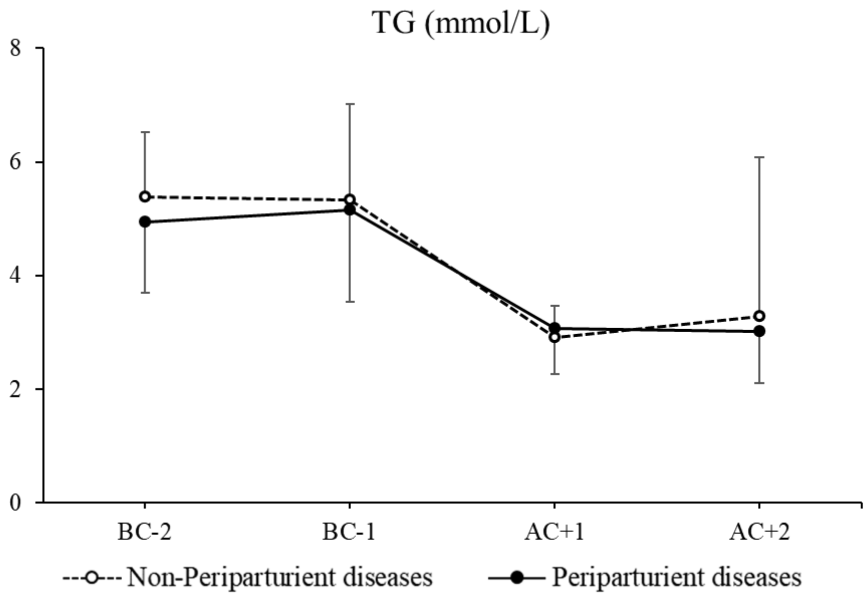

Fig. 4. Comparison of plasma triglycerides (TG) in cows with periparturient disease and non-periparturient disease at for four time periods. Data are expressed as means ± SD.

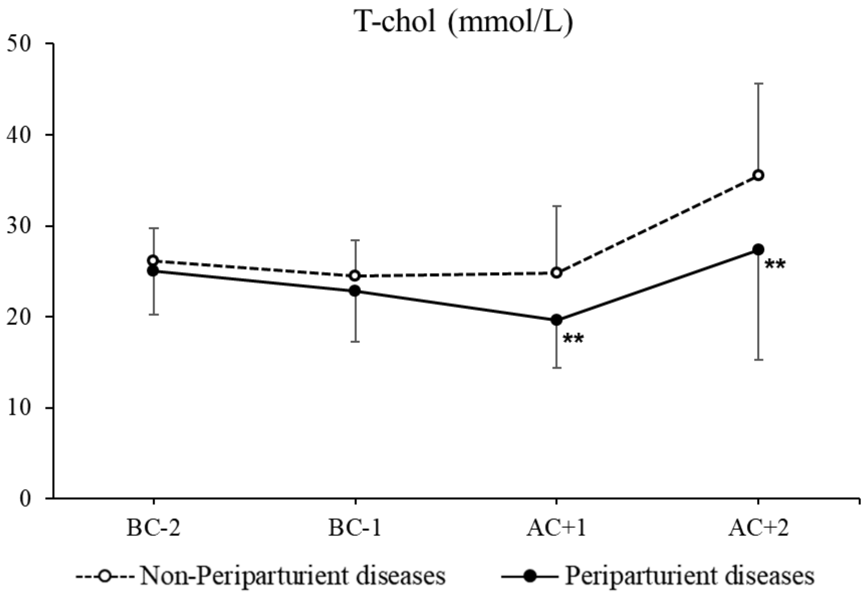

Fig. 5. Comparison of plasma total cholesterol (T-chol) in cows with periparturient disease and non-periparturient disease at for four time periods. Data are expressed as means ± SD. Significant difference between differences between periparturient disease cows and non-periparturient disease cows is indicated by *($p < 0.05$) and **($p < 0.01$).

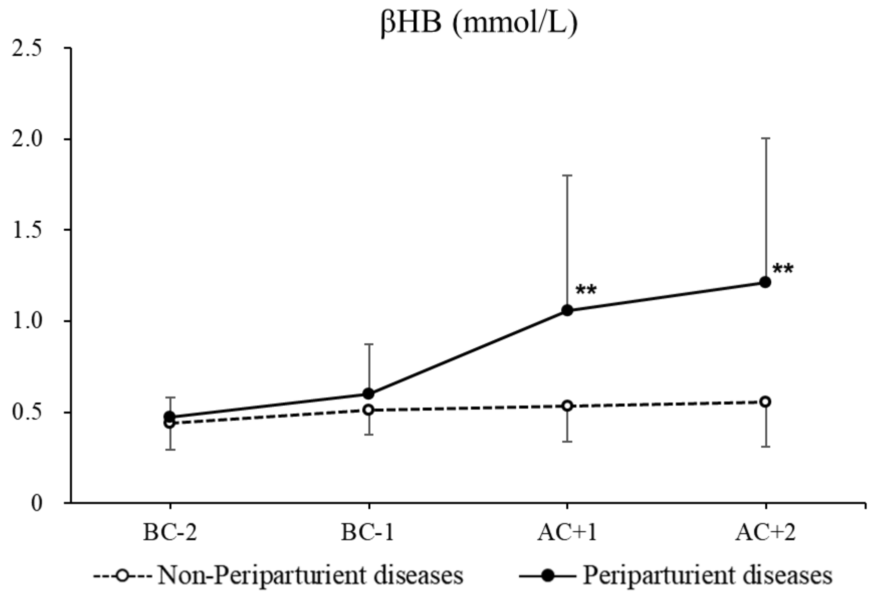

Fig. 6. Comparison of plasma β-hydroxybutyrate (βHB) in cows with periparturient disease and non-periparturient disease at for four time periods. Data are expressed as means ± SD. Significant difference between differences between periparturient disease cows and non-periparturient disease cows is indicated by *(p < 0.05) and **(p < 0.01).

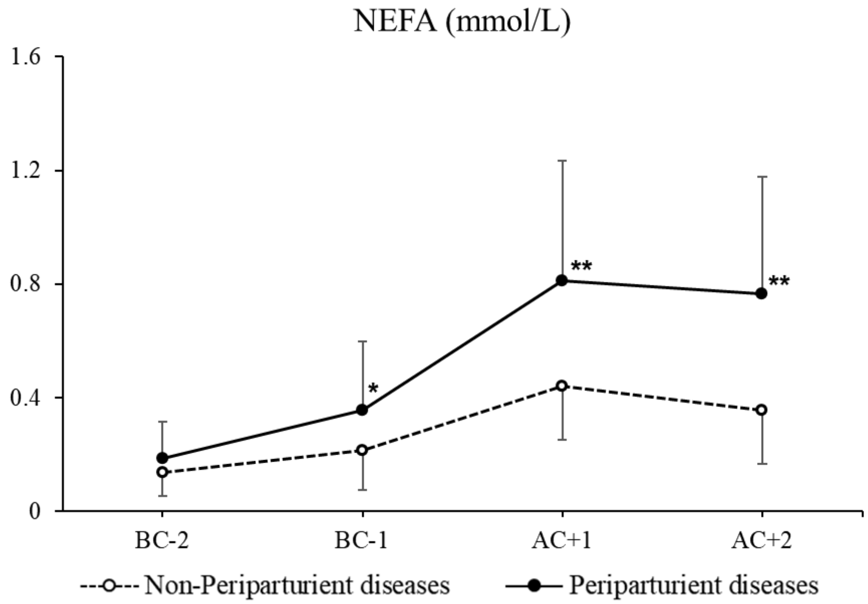

Fig. 7. Comparison of plasma non-esterified fatty acid (NEFA) in cows with periparturient disease and non-periparturient disease at for four time periods. Data are expressed as means ± SD. Significant difference between differences between periparturient disease cows and non-periparturient disease cows is indicated by *($p < 0.05$) and **($p < 0.01$).

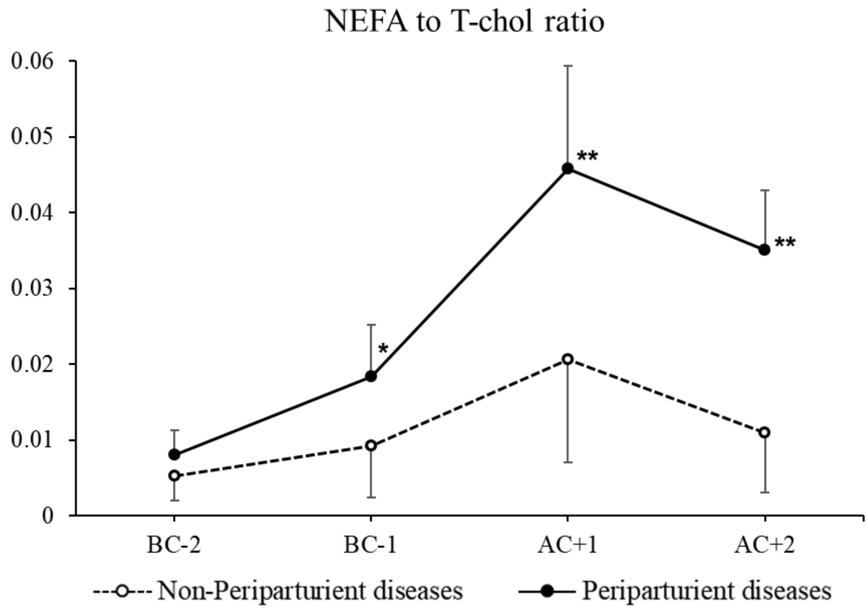

Fig. 8. Comparison of non-esterified fatty acid (NEFA) to total cholesterol (T-chol) ratio in cows with periparturient disease and non-periparturient disease at for four time periods. Data are expressed as means ± SD. Significant difference between differences between periparturient disease cows and non-periparturient disease cows is indicated by *(p < 0.05) and **(p < 0.01).

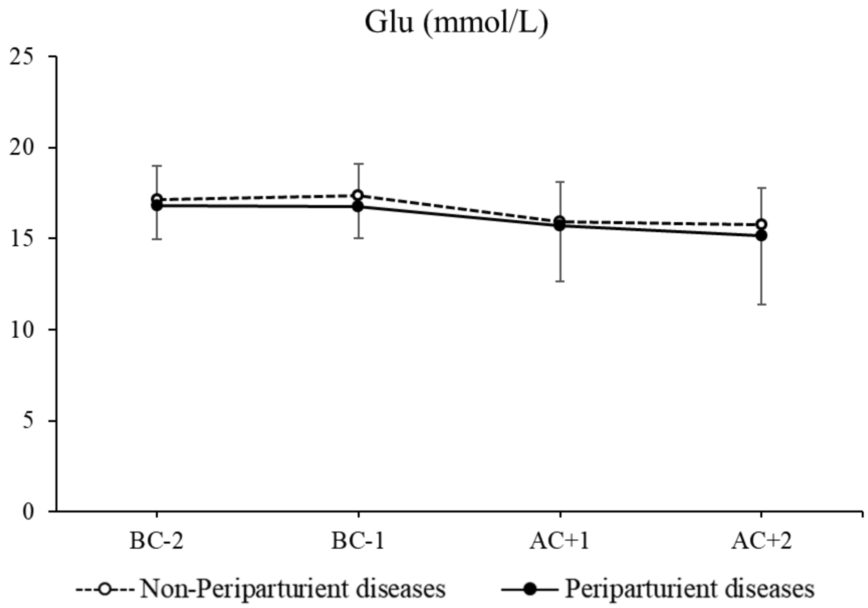

Fig. 9. Comparison of plasma glucose (Glu) in cows with periparturient disease and non-periparturient disease at for four time periods. Data are expressed as means ± SD.

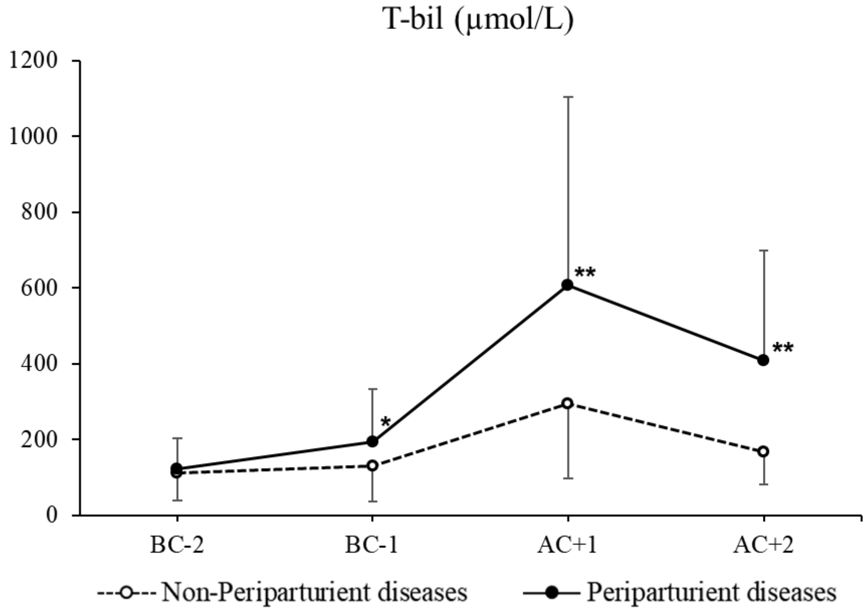

Fig. 10. Comparison of plasma total bilirubin (T-bil) in cows with periparturient disease and non-periparturient disease at for four time periods. Data are expressed as means ± SD. Significant difference between differences between periparturient disease cows and non-periparturient disease cows is indicated by *(p < 0.05) and **(p < 0.01).

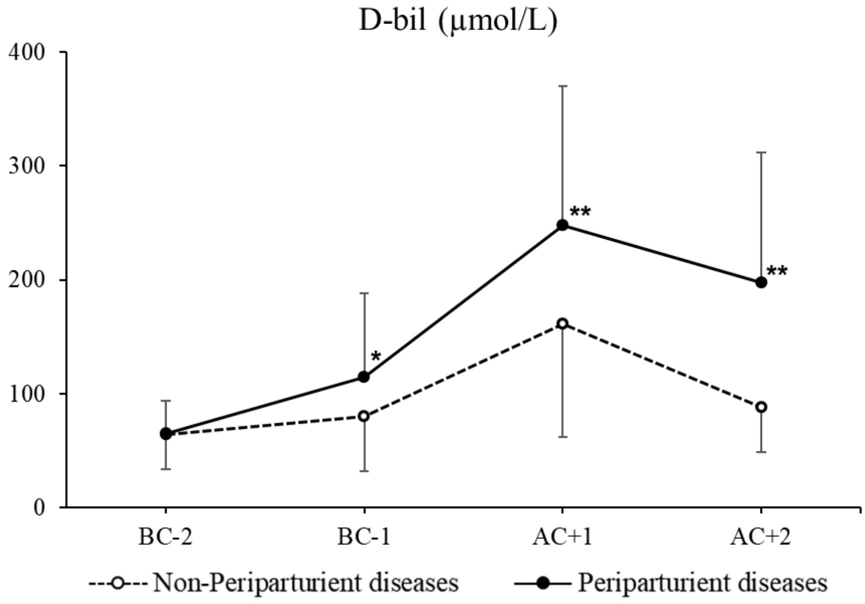

Fig. 11. Comparison of plasma direct bilirubin (D-bil) in cows with periparturient disease and non-periparturient disease at for four time periods. Data are expressed as means ± SD. Significant difference between differences between periparturient disease cows and non-periparturient disease cows is indicated by *(p < 0.05) and **(p < 0.01).

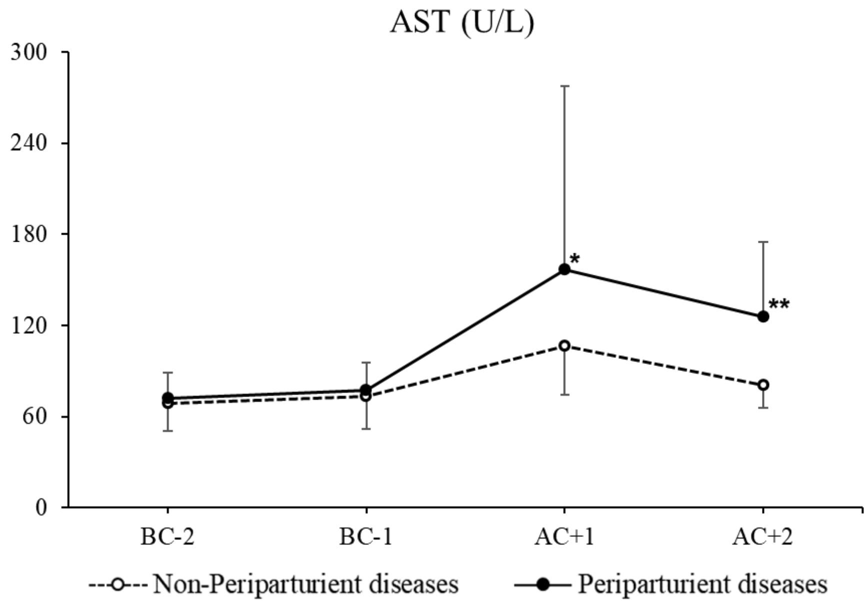

Fig. 12. Comparison of plasma aspartate aminotransferase (AST) in cows with periparturient disease and non-periparturient disease at for four time periods. Data are expressed as means ± SD. Significant difference between differences between periparturient disease cows and non-periparturient disease cows is indicated by *(p < 0.05) and **(p < 0.01).

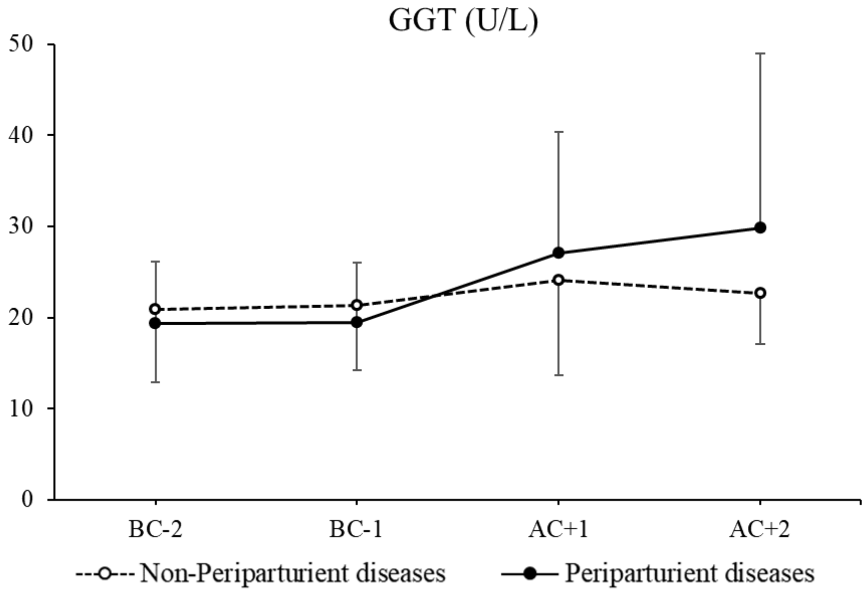

Fig. 13. Comparison of plasma γ-glutamyl transferase (GGT), calcium (Ca), and inorganic phosphate (iP) in cows with periparturient disease and non-periparturient disease at for four time periods. Data are expressed as means ± SD.

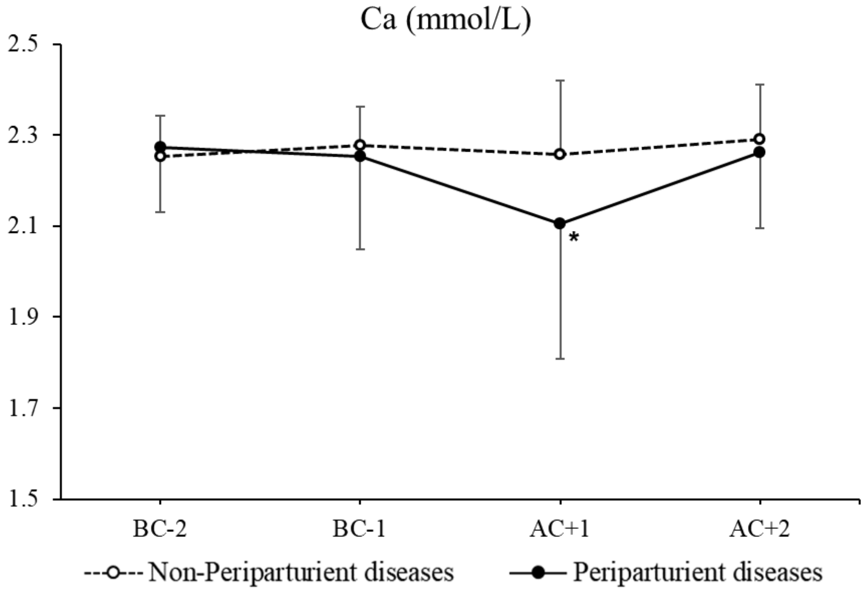

Fig. 14. Comparison of plasma calcium (Ca) in cows with periparturient disease and non-periparturient disease at for four time periods. Data are expressed as means ± SD. Significant difference between differences between periparturient disease cows and non-periparturient disease cows is indicated by *(p < 0.05) and **(p < 0.01).

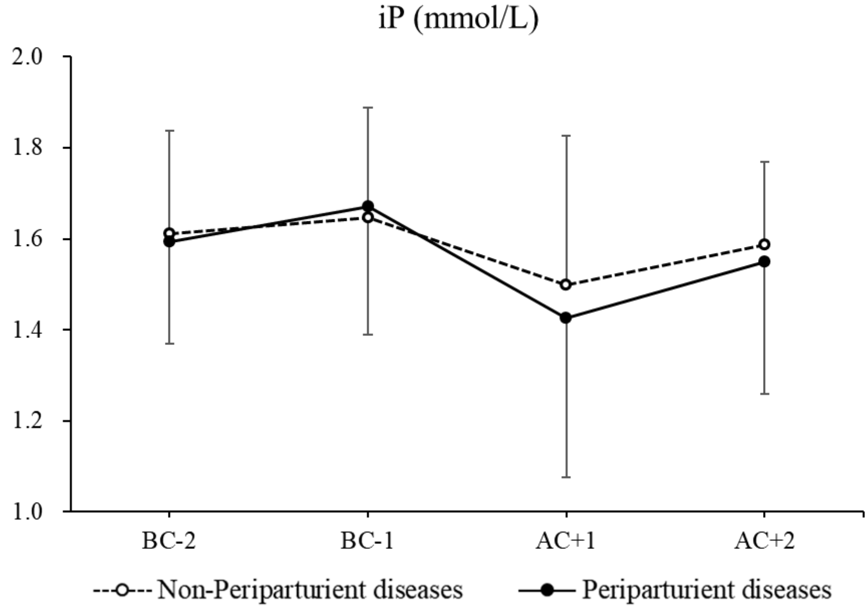

Fig. 15. Comparison of plasma inorganic phosphate (iP) in cows with periparturient disease and non-periparturient disease at for four time periods. Data are expressed as means ± SD.

2-2. ROC analysis

There were significant group differences in the levels of BCS, NEFA, T-bil, D-bil, and NEFA to T-chol ratio before parturition. These differences were evaluated using ROC analysis to determine a cut-off value for association with the occurrence of periparturient diseases (Table 4). Each parameter included in the ROC curve analysis was less accurate, as AUC was less than 0.7.

Table 4. Receiver operator characteristic (ROC) curve for the prepartum body condition score (BCS) at 15 to 30 days before calving (BC-2). The prepartum plasma non-esterified fatty acid (NEFA), total bilirubin (T-bil), and direct bilirubin (D-bil) concentrations and NEFA to T-chol ratio at 1 to 14 days before calving (BC-1) were measured as predictors of periparturient disease

Period	Para meter	Cut-off value	Sen	Spe	Standard error	AUC	+LR	95% CI
BC-2	BCS	≥ 3.44	80.6	48.1	0.074	0.643 $p = 0.061$	1.56	0.498-0.789
BC-1	NEFA (mmol/L)	≥ 0.39	45.2	85.2	0.068	0.697 $p = 0.010$	3.05	0.563-0.831
BC-1	T-bil (μmol/L)	≥ 150	61.3	74.1	0.072	0.683 $p = 0.017$	2.36	0.542-0.823
BC-1	D-bil (μmol/L)	≥ 85.5	64.5	70.4	0.072	0.670 $p = 0.026$	2.18	0.529-0.812
BC-1	NEFA to T-chol ratio	≥ 0.012	58.1	77.8	0.069	0.697 $p = 0.010$	2.61	0.563-0.831

Sen, sensitivity; Spe , specificity; AUC, area under the ROC curve; +LR, positive likelihood ratio; CI, confidence interval

2-3. Predictive models using logistic regression

ROC curves with NEFA and NEFA to T-chol ratio had the highest AUC. With BCS at BC-2, plasma T-bil and D-bil concentrations, and NEFA to T-chol ratio at BC-1 were calculated for the predictive models using logistic regression and the presence or absence of postpartum disease (Fig 16). The sensitivity, specificity, and AUC were determined as 71.0%, 77.8%, and 0.769, respectively (Table 5).

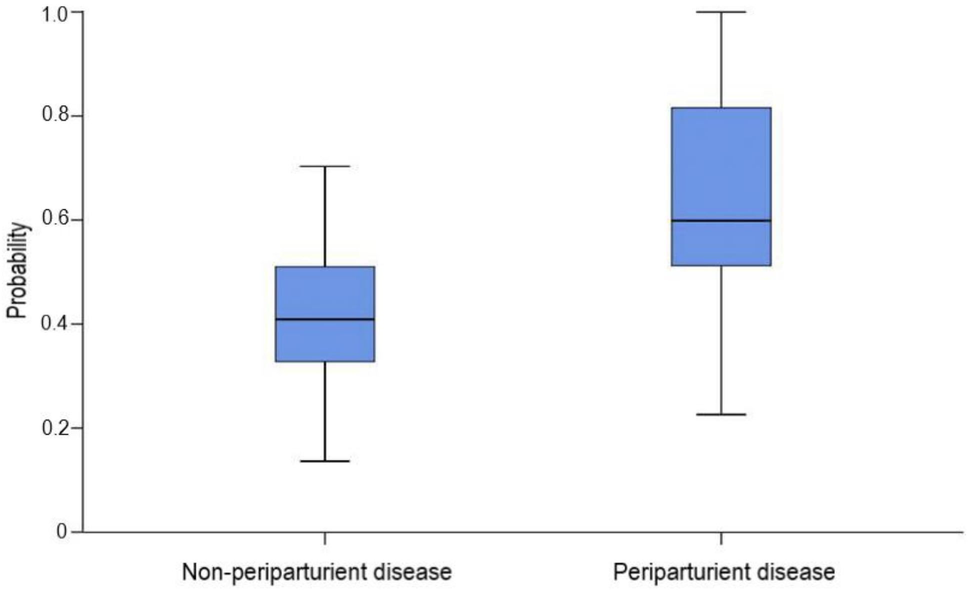

Fig 16. Comparison of diseased and non-diseased cows with regard to the predicted probability model using logistic regression analysis. Data are presented as mean ± SD and minimum and maximum values to non-periparturient disease and periparturient disease groups.

Table 5. Receiver operator characteristic (ROC) curve for to the predicted probability model using logistic regression analysis, that was measured as a predictor of periparturient disease

Predicted model	Cut-off value	Sen	Spe	Standard error	AUC	+LR	95% CI
$P = \frac{1}{1+e^{-z}}$	$\geq$ 0.523	71.0	77.8	0.063	0.769 p = 0.000	3.19	0.646-0.893

Sen=sensitivity, Spe=specificity, AUC=area under the ROC curve, +LR=positive likelihood ratio, CI=confidence interval

$z = -12.874 + 3.376X_1 - 0.013X_2 + 0.021X_3 + 103.893X_4$

X_1 = Body condition score (BCS) 15 to 30 days before calving (BC-2)

X_2 = Total bilirubin (T-bil) 1 to 14 days before calving (BC-1)

X_3 = Direct bilirubin (D-bil) BC-1

X_4 = Non-esterified fatty acid (NEFA) : total cholesterol (T-chol) ratio BC-1

3. Further statistical analysis

There was no significant difference in incidence rate according to the parity through chi-test (χ^2 = 0.07 and p = 0.792): nine of the 16 primiparous cows (56.3%) and 22 of the 42 multiparous cows (52.4%) had postpartum disease. In primiparous cows, there were no significant differences in the parameters between at least one disease and no-disease cows (BC-2 and BC-1). However, in multiparous cows, there was a significant difference with high values in BCS and NEFA at BC-2 (p = 0.008, p = 0.049, respectively) and high values in NEFA at BC-1 (p = 0.015). There was a significant difference in milk yield between diseased and non-diseased cows in both postpartum periods (AC+1 and AC+2). At AC+1, the mean milk yield for non-diseased cows was 26.1 ± 8.0 L (mean ± SD), and that for diseased cows was 19.2 ± 9.0 L (p = 0.009). In AC+2, the mean milk yield for non-diseased cows was 33.1 ± 6.5 L and that of for diseased cows was 21.6 ± 12.3 L (p = 0.000).

The incidence of subclinical, clinical and no disease was 26, 13, and 27 cases from 58 Holstein parturient cows. Nineteen of the 31 cows with at least one disease had one disease, and 12 of the 31 cows had two or more diseases. A significant difference between subclinical vs clinical vs non-disease with prepartum blood biochemical parameters were only in the case of NEFA, in which subclinical cases had significantly higher NEFA concentrations at BC-1 than no-disease cases (*p* = 0.038). Moreover, the levels of BCS and Ca of multiple disease cases was lower than that of one disease cases at BC-1 (*p* = 0.019, *p* = 0.007, respectively).

Discussion

In this study, 48.9% of cows experienced one or more diseases after calving. This is consistent with several studies showing that as much as 50% of cows experience one or more periparturient diseases in conventional farms [LeBlanc *et al.* 2005, Sepúlveda-Varas *et al*. 2015, Van Saun *et al.* 2006]. The incidence of ketosis (23.4% for subclinical ketosis and 6.4% for clinical ketosis) and displaced abomasum (10.6%) in this study were higher than in those from two conventional farm studies in Korea [Jeong *et al.* 2015, Jeong *et al*. 2019]. However, the incidence of retained placenta (13.8%) was lower than that reported in the aforementioned two studies (30.0% and 30.4%) [Jeong *et al.* 2015, Jeong *et al*. 2019]. In view of these results, by increasing the feeding amount from 1 week before parturition on this organic farm, retained placenta that checked within 24h after calving may be less. However, as it progresses to the NEB state, it may be judged that the incidence of ketosis and displaced abomasum is high due to the limitation of organic supplementary feed to supplement the insufficient energy for peak of milk yield in the early lactation period.

When the incidence of disease of primiparous and multiparous cows were compared with the incidence of disease of 94 Holstein parturient cows, subclinical ketosis was the most common, followed by subclinical hypocalcemia in three cases. However, there was a difference in the incidence rate of displaced abomasum. The incidence rate of displaced abomasum of primiparous cows was 20%, which was twice as high as that of multiparous cows (7.2%) and that of all parturient cows (10.6%). As a result of this, it is possible that the disease control of the primiparous cows was difficult due to the poor maintenance of the breeding management of the heifers during this period investigated at this organic farm.

Prepartum blood data between cows with one disease and multiple diseases were significantly different for Ca in this study. Periparturient diseases are not single entities but are interconnected [Van Saun *et al.* 2006]. Indeed, cows with hypocalcemia are more likely to develop displaced abomasum, ketosis, and retained placenta [Curtis *et al*. 1983, Goff *et al*. 2008, Goff *et al.* 1997]. Eleven of 12 cows with multiple diseases showed subclinical hypocalcemia in the present study. Subclinical hypocalcemia may result in decreased intestinal motility due to decreased muscle contractility, which may lead to decreased appetite. In other words, it increases the simultaneous risk of displaced abomasum, ketosis and even hepatic lipidosis. Thus, subclinical hypocalcemia is a significant health risk for dairy cows and should be taken preventive measures to reduce potential economic losses to farms.

One study found that a lower BCS in primiparous cows during peripartum influences the occurrence of postpartum health problems [Wang et al. 2019]. However, other studies have shown that dry cows with a better body condition had a greater depression of DMI and a deeper NEB than cows with a lower body condition in the peripartum period [Rukkwamsuk et al. 1999]. According to the results of this study, cows with at least one disease had a higher BCS at BC-2, and when cows with one disease and multiple diseases were compared, cows with multiple diseases had significantly lower BCS in BC-1. Through these studies, prepartum BCS screening itself is vital in predicting the disease, but the degree of prepartum decrease in BCS also seems valuable.

Several studies have indicated that monitoring prepartum NEFA concentration is crucial for predicting postpartum diseases such as displaced abomasum [LeBlanc et al. 2005, Ospina et al. 2010], retained placenta [Quiroz-Rocha et al. 2009], and ketosis [Ospina et al. 2010]. In this study, there was a significant difference between no disease and subclinical disease cows, and between cows with at least one disease and cows with non-disease in the case NEFA in BC-1. In the case of multiparous cows, cows with at least one disease had a higher NEFA than cows without disease at BC-2 and BC-1. In view of these results, an increase in NEFA, which reflects the state of NEB, is an important indicator in predicting postpartum disease before calving rather than other parameters.

Elevated NEFA concentrations convert to TG, which must bind to VLDL to be transported to the liver [Puppel *et al*. 2016, Reid *et al*. 1977]. Low cholesterol levels limit VLDL production and TG accumulates in the liver, increasing the potential for fat infiltration [Herdt *et al.* 2000, Katoh *et al*. 2002]. Hence, a decrease in plasma T-chol concentration may indicate a decreased DMI and lead to fatty liver due to a decrease in TG transport. Some investigators have suggested the need to assess the NEFA to T-chol ratio for the association between NEFA and T-chol [Holtenius *et al*. 1990, Kaneene *et al.* 1997]. The NEFA to T-chol ratio of postpartum diseased cows was significantly higher than that of non-postpartum diseased cows at BC-1 in the present study (p < 0.05). A previous study reported that the accumulation of fat and changes in cytoplasmic organelles in the liver of fasted cows was accompanied by impaired hepatocyte function, as indicated by the elevated serum T-bil concentration and AST activity [Reid *et al*. 1977]. Another study also showed that in cows with hyperbilirubinemia, the frequently described signs were anorexia and rumen stasis [McSherry *et al*. 1984]. Hyperbilirubinemia has also been observed in cattle with fatty liver associated with ketosis [Puppel *et al.* 2016]. Bilirubin concentrations were significantly higher in cattle with hepatic lipidosis than in those with non-fatty liver [Mostafavi *et al.* 2013]. It is believed that increase in T-bil and D-bil is related to a decrease in the hepatic absorption of bilirubin due to competition with other anions such as free fatty acids and an impaired hepatocyte function [McSherry *et al*. 1984]. My findings regarding the association of high NEFA and T-bil concentrations with the postpartum metabolic disease before calving are consistent with those of previous studies [Puppel *et al.* 2016, Reid *et al*. 1977]. Starting with the increase in NEFA, which reflects the status of NEB, which showed a significant difference between subclinical disease and the absence of disease, changes in various blood levels can be viewed as related rather than independently increasing or decreasing levels.

To predict the risk of developing postpartum disease, the cutoff values of prepartum BCS, NEFA, NEFA to T-chol ratio, T-bil and D-bil of dairy cows were determined using ROC curve analysis. However, there no parameter demonstrated an AUC greater than 0.7. ROC analysis based on logistic regression with all parameters with significant differences revealed a higher AUC (0.769), sensitivity (71.0%), and specificity (77.8%). One study confirmed that the results of an ROC analysis with a logistic regression model that combined milk yield and composition for the presence or absence of ketosis had a higher AUC than that of one composition [Kayano *et al*. 2015]. In another study, a high level of accuracy was achieved using a predictive model that combined multiple variables to predict calving time [Fadul *et al*. 2017]. It is suggested that better results that disease can be prevented can be obtained by analyzing each parameter and developing a predictive model by multiplying the logistic regression coefficients according to the negative and positive correlations of the parameters for the presence or absence of postpartum metabolic disease, which is a better direction than predicting postpartum disease through each variable.

Conclusion

In conclusion, this study investigated the incidence rate of postpartum metabolic diseases in dairy cows in an organic farm. I confirmed the presence of significant differences between the diseased and non-diseased group in prepartum BCS, NEFA, T-bil, D-bil, and the NEFA to T-chol ratio. ROC analysis using these parameters could be used to predict disease occurrence with moderate accuracy. However, this study was conducted on only one organic farm, and since the number of individuals used in the study is small, additional research is needed to increase the number of individuals and farms to create an index that can be applied to various farms.

Conflict of Interests

The author declares that there is no conflict of interest.

References

Asl AN, Nazifi S, Ghasrodashti AR, Olyaee A. Prevalence of subclinical ketosis in dairy cattle in the Southwestern Iran and detection of cutoff point for NEFA and glucose concentrations for diagnosis of subclinical ketosis. Preventive Veterinary Medicine. 2011;100(1): 38-43

Bell AW. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. Journal of Animal Science. 1995;73(9):2804-2819

Caixeta LS, Omontese BO. Monitoring and improving the metabolic health of dairy cows during the transition period. Animals. 2021;11(2):352

Chamberlin WG, Middleton JR, Spain JN, Johnson GC, Ellersieck MR, Pithua P. Subclinical hypocalcemia, plasma biochemical parameters, lipid metabolism, postpartum disease, and fertility in postparturient dairy cows. Journal of Dairy Science. 2013;96(11): 7001-7013

Curtis CR, Erb HN, Sniffen CJ, Smith RD, Powers PA, Smith MC, et al. Association of parturient hypocalcemia with eight periparturient disorders in Holstein cows. Journal of the American Veterinary

Medical Association. 1983;183(5):559-561

Faul M, Bogdahn C, Alsaaod M, Husler J, Starke A, Steiner A, et al. Prediction of calving time in dairy cattle. Animal Reproduction Science. 2017;187:37-46

Ferguson JD, Galligan DT, Thomsen N. Principal descriptors of body condition score in Holstein cows. Journal of Dairy Science. 1994;77(9):2695-2703

Goff JP. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. Veterinary Journal. 2008;176(1):50-57

Goff JP, Horst RL. Physiological changes at parturition and their relationship to metabolic disorders. Journal of Dairy Science. 1997b;80(7):1260-1268

Greiner M, Pfeiffer D, Smith RD. Principles and practical application of the receiver operating characteristic analysis for diagnostic tests. Preventive Veterinary Medicine. 2000;45(1-2):23-41

Grummer RR. Etiology of lipid-related metabolic disorders in periparturient dairy cows. Journal of Dairy Science. 1993;76(12):3882-3896

Grummer RR. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. Journal of Animal Science. 1995;73(9):2820-2833

Herdt TH. Ruminant adaptation to negative energy balance. Influences on the etiology of ketosis and fatty liver. Veterinary Clinics of North America: Food Animal Practice. 2000;16(2):215-230

Holtenius P, Hjort M. Studies on the pathogenesis of fatty liver in cows. Bovine Practitioners. 1990;25:91-94

Hosmer DW, Lemeshow S. ln: *Statistics in Medicine*. 2**nd** ed. New York: Wiley; 2000, Chapter 1, Applied Logistic Regression; 1-10

Jeong JK, Choi IS, Kang HG, Jung YH, Hur TY, Kim IH. Postpartum reproductive tract recovery and prevalence of health problems in dairy cows. Journal of Veterinary Clinic. 2015;32(2):168-173

Jeong JK, Hur TY, Jung YH, Kang HG, Kim IH. Identification of predictive biomarkers of peri- and postpartum disorders in dairy cows. Korean Journal of Veterinary Research. 2019;59(1):1-8

Kaneene JB, Miller R, Herdt TH, Gardiner JC. The association of serum nonesterified fatty acids and cholesterol, management and feeding practices with peripartum disease in dairy cows. Preventive Veterinary Medicine. 1997;31(1-2):59-72

Katoh N. Relevance of apolipoproteins in the development of fatty liver and fatty liver-related peripartum diseases in dairy cows. Journal of Veterinary Medical Science. 2002;64(4):293-307

Kayano M, Kataoka T. Screening for ketosis using multiple logistic regression based on milk yield and composition. The Journal of Veterinary Medical Science. 2015;77(11):1473-1478

LeBlanc S. Monitoring metabolic health of dairy cattle in the transition period. Journal of Reproduction and Development. 2010;56 Suppl: S29-S35

LeBlanc SJ, Leslie KE, Duffield TF. Metabolic predictors of displaced abomasum in dairy cattle. Journal of Dairy Science. 2005;88(1):159-170.

McArt JAA, Nydam DV, Oetzel GR. Epidemiology of subclinical ketosis in early lactation dairy cattle. Journal of Dairy Science. 2012;95(9):5056-5066

Mcsherry BJ, Lumsden JH, Valli VE, Baird JD. Hyperbilirubinemia in sick cattle. Canadian Journal of Comparative Medicine. 1984;48(3):237-240

Mostafavi M, Seifi HA, Mohri M, Jamshidi A. Optimal thresholds of metabolic indicators of hepatic lipidosis in dairy cows. Revue de Medecine Veterinaire. 2013;164(12):564-571

Ospina PA, Nydam DV, Stokol T, Overton TR. Evaluation of nonesterified fatty acids and beta-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. Journal of Dairy Science. 2010;93(2):546-554

Puppel K, Kuczynska B. Metabolic profiles of cow's blood; a review. Journal of the Science of Food and Agriculture. 2006;96(13):4321-4328

Quiroz-Rocha GF, LeBlanc S, Duffield T, Wood D, Leslie KE, Jacobs RM. Evaluation of prepartum serum cholesterol and fatty acids concentrations as predictors of postpartum retention of the placenta in dairy cows. Journal of the American Veterinary Medical Association. 2009;234(6):790-793

Reinhardt TA, Lippolis JD, McCluskey BJ, Goff JP, Horst RL. Prevalence of subclinical hypocalcemia in dairy herds. Veterinary Journal. 2011;188(1):122-124

Reid IM, Harrison RD, Collins RA. Fasting and refeeding in the lactating dairy cow: 2. The recovery of liver cell structure and function following a six-day fast. Journal of Comparative Pathology. 1977;87(2):253-265

Ro Y, Choi W, Kim H, Kim D. Prepartal decrease in plasma total cholesterol concentration in dairy cows developed subclinical ketosis. Journal of Veterinary Clinics. 2017;34(3):222-224

Rukkwamsuk T, Kruip TA, Wensing T. Relationship between overfeeding and overconditioning in the dry period and the problems of high producing dairy cows during the postparturient period. Veterinary Quarterly. 1999;21(3):71-77

Sepulveda-Varas P, Weary DM, Noro M, von Keyserlingk MAG. Transition diseases in grazing dairy cows are related to serum cholesterol and other analytes. PLoS ONE. 2015;10(3):e0122317

Van Saun RJ. Metabolic profiles for evaluation of the transition period. American Association of Bovine Practitioners. 2006;39:130-138

Wang Y, Huo P, Sun Y, Zhang Y. Effects of body condition score changes during peripartum on the postpartum health and production performance of primiparous dairy cows. Animals. 2019;9(12):1159

국 문 초 록

한국의 유기농 목장에서 분만 후 대사성 질병의 예측을 위한 ROC 분석

지도교수 김 단 일

김 도 희

서울대학교 대학원
수의학과 산업동물임상의학 전공

유기농 목장에서 분만 전 생산성 손실을 예방하려면 분만 후 질병을 예측해야 한다. 본 연구의 목적은 국내 유기농 목장에서 분만 후 대사성 질병의 발병률을 조사하고, 질병과 분만 전 혈액 생화학적 매개변수와의 연관성을 확인하고, 이들 매개변수의 정확도를 ROC(Receiver Operating Characteristic) 분석으로 질병에 취약한 소를 예측하는 것이다.

데이터는 유기농 목장에서 2년 동안 분만한 94마리의 홀스타인 젖소 (25마리의 초산 및 69마리의 다산) 중 58마리의 홀스타인 젖소 (16마리의 초산 및 42마리의 다산)에서 수집되었다. 분만 전 4주에서 분만 후 4주까지의 전이기 동안에 신체검사와 함께 2주마다 채혈을 통해 혈액 생화학을 시행하였다.

94마리의 분만한 젖소 중 46마리(48.9%)의 젖소가 적어도 하나의 분만 후 질병으로 진단되었다. 각 발생률은 준임상형 케톤증이 23.4%, 준임상형 저칼슘혈증이 19.1%, 태반정체가 13.8%, 4위 전위가 10.6%, 임상형 케톤증이 6.4%였다.

분만한 58마리의 홀스타인 젖소의 데이터를 하나이상의 질병이 있는 젖소 31마리와 질병이 없는 젖소 27마리, 두 그룹으로 나누었다. 적어도 하나의 질병이 있는 경우와 질병이 없는 경우 사이에 신체 상태 점수 (BCS), 비에스테르화 지방산 (NEFA), 총 빌리루빈 (T-bil), 직접 빌리루빈 (D-bil) 및 NEFA 대 총 콜레스테롤(T-chol) 비율에서 분만 전 수준에서 유의한 차이가 있었다 ($p < 0.05$). 이러한 분만 전 매개변수 각각에 대한 ROC 분석은 곡선 아래 면적 (AUC) < 0.7을 나타냈다. 그러나 이러한 모든 매개변수를 포함하는 로지스틱 회귀 분석을 사용한 ROC 분석에서는 민감도 (71.0%), 및 특이도 (77.8%), AUC(0.769)를 나타냈다.

결론적으로, 분만 전 BCS, T-bil, D-bil 및 NEFA 대 T-chol 비율을 포함한 로지스틱 회귀 분석을 사용한 ROC 분석을 사용하여 중증도의 정확도로 분만 후 질병에 취약한 젖소를 예측할 수 있다.

주요어: 유기농 목장, 분만 후 질병, 대사성 파라미터, ROC 분석, 로지스틱 회귀분석

학번: 2020-22928