Kafkas Univ Vet Fak Derg
22 (2): 191-195, 2016
DOI: 10.9775/kvfd.2015.14065

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

Critical Thresholds of Nonesterified Fatty Acids and β-hydroxybutyrate in Transition Dairy Cows for Prediction of First Service Conception Rate

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Article Code: KVFD-2015-14065 Received: 18.07.2015 Accepted: 18.01.2016 Published Online: 19.01.2016

Abstract

The objective of this study was to establish cow level critical thresholds for β-hydroxybutyrate (BHBA) and nonesterified fatty acids (NEFA) to predict conception to first service. The data were generated from 97 Holstein cows (2 to 5 parities) on a large commercial farm. Serum concentrations of BHBA and NEFA were measured in all cows on day 10 prepartum and, weeks 1, 2, 4 and 6 postpartum. NEFA and BHBA analyzed with receiver operator characteristic (ROC) analysis to determine critical thresholds for predicting pregnancy to first service. NEFA in weeks 4 and 6 postpartum were the only significant predictors identified in the ROC analysis. Optimum critical thresholds for NEFA in weeks 4 and 6 were 201.15 µmol/L and 203.4 µmol/L, respectively (P<0.05). The critical threshold for serum BHBA in the prepartum cohort was 600 µmol/L (P=0.1), which predicted conception to first service. Logistic regression analysis indicated that the risk of conceiving was 82.4% and 88.6% lower for cows with NEFA ≥201.15 in week 4 (OR=0.176; P=0.001) and NEFA ≥203.4 µmol/L in week 6 (OR=0.114; P=0), respectively. In conclusion, NEFA concentrations within 4 and 6 weeks after calving were associated with lower probability of pregnancy at the first AI.

Keywords: *Dairy cow, Nonesterified fatty acids, β-hydroxybutyrate, First service conception*

Geçiş Dönemi Sütçü İneklerde İlk Tohumlamada Gebe Kalma Oranını Tahmin Etmede Esterlenmemiş Yağ asitleri ve β-hidroksibütirat Kritik Eşik Değerleri

Özet

Bu çalışmanın amacı ineklerde ilk tohumlamada gebe kalma oranını tahmin etmede β-hidroksibütrat (BHBA) ve esterlenmemiş yağ asitleri (NEFA) kritik eşik değerlerini belirlemektir. Çalışmanın verileri ticari büyük bir çiftlikte 97 adet Holstein ırkı inekten (2 ile 5 parite) elde edildi. Tüm ineklerde BHBA ve NEFA serum konsantrasyonları prepartum 10. günde ve postpartum 1, 2, 4 ve 6. haftalarda ölçüldü. BHBA ve NEFA ilk tohumlamada gebe kalma eşik değerini belirlemek amacıyla Receiver Operator Characteristic (ROC) ile analiz edildi. Postpartum 4. ve 6. haftalardaki NEFA ROC analizinde belirlenen tek anlamlı değerlerdi. 4. ve 6. haflarda NEFA için optimal kritik eşik değerleri sırası ile 201.15 µmol/L ve 203.4 µmol/L olarak tespit edildi (P<0.05). Prepartum grubunda serum BHBA için kritik eşik 600 µmol/L (P=0.1) olarak belirlendi. Lojistik regresyon analizi ile gebe kalma riski 4. haftada NEFA ≥201.15 ineklerde (OR=0.176; P=0.001) %82.4 ve 6. haftada NEFA ≥203.4 µmol/L ineklerde (OR=0.114; P=0) %88.6 daha düşük olarak belirledi. Sonuç olarak, doğumdan sonra 4 ve 6. haftalarda NEFA konsantrasyonları ilk suni tohumlamada gebe kalma olasılığı ile düşük derecede ilişkilidir.

Anahtar sözcükler: *Süt ineği, Esterlenmemiş yağ asitleri, β-hidroksibütrat, İlk tohumlamada gebelik*

INTRODUCTION

Most transition dairy cows enter a state of negative energy balance (NEB) for three important reasons: 1) increased energy demands at parturition, 2) decreased DMI shortly before parturition, and 3) lagging DMI compared with energy demands due to milk production [1,2].

Body fat stores are mobilized into the bloodstream in the form of nonesterified fatty acids (NEFAs) because of increased energy demand and decreased DMI and contribute

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to overall energy requirements during early lactation, some of which is taken up by the liver. In the liver, some NEFA are oxidized or re-esterified into triglycerides that are either exported as very low density lipoproteins or stored in the liver. During the periparturient period, high rates of NEFA enter the liver and sometimes exceed the liver's capacity to secrete triglycerides as very low density lipoproteins, leading to an accumulation of triglycerides [3]. Increased amounts of NEFA that are removed by the liver control ketogenesis and thus, β-hydroxybutyrate (BHBA) production [4].

At the cow level, increased BHBA and NEFA concentrations have been used as markers of excessive NEB. Previous studies have indicated that increased concentrations of these metabolites are related to increased risk of developing detrimental health [5,6], reproductive [7,8], and production outcomes [9].

The aims of this research are the followings: 1) to specify whether concentrations of BHBA and NEFA measured at 10 days prepartum and in each of the first, second, fourth and sixth weeks postpartum could be used at herd level to predict success of conception to first service and in which times relative to calving were most effective in predicting fertility; 2) to determine the cutoff point of NEFA and BHB concentrations for diagnosis of conception using receiver operating characteristic (ROC) analysis.

MATERIAL and METHODS

Study Population and Design

The study was conducted on 97 lactating Holstein cows of parities two to five in a large commercial dairy herd, in Chaharmahal and Bakhtiari province of Iran. In this study, cows have fed a TMR-based diet (All diets were based on alfalfa, corn silage, and a combination of concentrate including corn, soya meal and bone meal).

Seasonal effects were minimized as most of the cows on farm calved during a one-hundred-day period from August until November in 2014. Blood samples were collected at 5 to 6 a.m. (before feeding) on day 10 prepartum and weeks one, two, four and six postpartum, via the tail vein into a glass tube.

Blood samples were left to clot at room temperature for about thirty minutes and then centrifuged at 2.000×g. The obtained serum samples were kept at -20°C until analyzed for BHBA and NEFA concentrations. These metabolites were determined by a D-3-hydroxybutyrate kit and a NEFA Kit (Randox Laboratories Ltd, Ardmore, UK). The cows were inseminated by an expert inseminator when standing heat was observed. Pregnancy diagnosis was performed by ultrasonography 30 to 40 days after service and the second palpation was done two weeks later to validate the pregnancy.

Statistical Analysis

Receiver Operator Characteristic (ROC) Analysis for Critical Thresholds: In this study, BHB and NEFA in different times were evaluated with receiver operator characteristic (ROC) analysis in order to determine critical thresholds for predicting conception.

The ROC curves analyze sensitivity versus 100 - specificity. Sensitivity was the proportion of animals conceived at first service that were below a given metabolite threshold, and specificity was the proportion of animals that did not conceive that was above a given threshold [10].

The point on the ROC curve that had the highest combined sensitivity and specificity was considered the critical threshold. In this analysis, there is an area under the curve (AUC) and a P-value for each parameter in different times. The value of P indicates if this parameter is an appropriate indicator for prediction of conception or not? Interpretation of this critical threshold was based on the area under the curve (AUC) such that if the AUC=0.5, it was noninformative; if $0.5 < AUC \leq 0.7$, it was accurate; if $0.7 < AUC \leq 0.9$, it was very accurate; if $0.9 < AUC < 1$, it was highly accurate; and if AUC = 1, then it was considered perfect [11].

Logistic regression: The odds ratios (OR) of conception to first service outcome given NEFA or BHBA concentrations were modeled with multivariable regression techniques, accounting for clustering of cows within herds.

Univariable analyses were first performed to assess the association between pregnancy at the first AI and categorical cow-level covariates (calf sex, calf weight, parity, BCS, BCS loss from calving to first service, postcalving clinical disease, and occurrence of dystocia) with t-test as a random effect. Parity was categorized into 2 and more than 2. Body condition score loss was categorized as less than one unit and one or more than one unite. A binary disease variable was created and coded 1 if a cow was diagnosed with dystocia, retained placenta, metritis, endometritis, ovarian cyst or at least one of these disease before 30 DIM. Variables that were not associated with pregnancy at the first AI in the univariable analysis (P>0.0.05) were not considered further.

For each significant metabolite and week of sampling in ROC analysis, dichotomized metabolites concentrations based on determined thresholds and significant covariates in the univariable analysis were submitted to multivariable logistic regression, using a binary distribution. The predicted probabilities of pregnancy were estimated from the model.

RESULTS

Conception rate to first service was 29% (28/97). The present study revealed several relationship estimates

between traits and fertility that were statistically significant (P<0.05) or tended to be significant (P=0.1).

Parity and body condition score loss from calving to first service were associated with the odds of pregnancy at first AI in univariable analyses whereas sex, weight, the mean of BCS, postcalving clinical disease and dystocia were not significantly different between pregnant and not pregnant cows after first service. First service conception rate progressively decreased from 33.7% for cows losing <1 unit of BCS to 10% for cows losing ≥1 unit of BCS from calving to first service (P=0.03). Also when cows were divided in to parity 2 (n=54) and ≥3 (n=43), cows with second parity had significantly higher conception rate compared with older cows (37% vs. 18%; P<0.05).

Critical Thresholds

NEFA and BHBA analyzed with ROC curves to determine the cow-level critical thresholds (combined highest sensitivity and specificity) to predict conception to first service. Non-esterified fatty acids in weeks 4 and 6 postpartum, was the only significant predictor identified in the ROC analysis. Tabular results of ROC curve determination of critical BHB and NEFA thresholds (μmol/L) for the prediction of conception are in *Table 1* and *2*. In summary, optimum critical threshold that had the highest combined sensitivity and specificity, for prepartum BHB was 600 μmol /L (P=0.1) and for NEFA in weeks 4 and 6 were 201.15 μmol/L (P=0.04) and 203.4 μmol/L (P=0.01), respectively, with 16.5, 51.5 and 49.5% of the animals at or above the threshold. All metabolite concentrations below these thresholds were associated with higher reproduction performance (i.e., cows with NEFA concentrations below related thresholds most likely conceived than cows with concentrations at or above the same thresholds).

Measures of Association

Odds ratios (OR) were calculated based on critical thresholds determined by ROC analysis. When NEFA was evaluated as the only main predictor (i.e., without BHB in the model) and after controlling for parity and BCS loss, the odds of conceiving were 82.4% and 88.6% lower for cows with NEFA ≥201.15 (OR=0.176; P=0.001) and NEFA ≥203.4 μmol/L (OR=0.114; P=0) in weeks 4 and 6 postpartum, respectively.

DISCUSSION

This study was an analysis of the association of serum metabolites in the transition period with early lactation reproductive performance on commercial dairies. This study was done in a commercial dairy farm with about 1450 lactating dairy cows in a mountainous area in Iran. Conception rate to first service on our farm was 29% which was the same as that of (27%) reported recently for 87 Iranian dairy cows by Kadivar et al.[12].

The results showed no significant association between BHBA concentrations and the odds of pregnancy at first AI. However, as far as we know, this is the first study which has reported an association (P=0.1) between precalving BHBA and reproductive performance. The optimal threshold of ≥600 μmol/L for predicting a reduction in reproductive performance was the same as that associated with a decreased milk yield in Chapinal et al.[13]. This finding is

Table 1. *Receiver operator characteristic curve determination of critical BHB thresholds as predictors of conception in transition dairy cows*
Tablo 1. *Geçiş dönemi ineklerde gebe kalma tahmini amacıyla kritik BHB eşik değerleri Receiver operator characteristic eğrisinin belirlenmesi*

Pregnancy Status	Critical Threshold[1]	Se[2]	Sp[3]	AUC[4]	P-value
Before parturition	421.55	53.6	30.4	0.605	0.1
Week 1	621.05	53.6	47.8	0.497	0.9
Week 2	480.7	53.6	42	0.502	0.9
Week 4	453.35	64.3	47.8	0.452	0.4
Week 6	468	60.7	47.8	0.487	0.8

*[1] Highest combined specificity (Sp) and sensitivity (Se), μmol/L; [2] **Se** = epidemiologic sensitivity; [3] **Sp** = epidemiologic specificity; [4] **AUC** = area under the curve*

Table 2. *Receiver operator characteristic curve determination of critical NEFA thresholds as predictors of conception in transition dairy cows*
Tablo 2. *Geçiş dönemi ineklerde gebe kalma tahmini amacıyla kritik NEFA eşik değerleri Receiver operator characteristic eğrisinin belirlenmesi*

Pregnancy Status	Ccritical Threshold[1]	Se[2]	Sp[3]	AUC[4]	P-value
Before parturition	133.5	57.1	47.8	0.486	0.8
Week 1	673.95	57.1	53.6	0.495	0.9
Week 2	466.65	60.7	46.4	0.490	0.8
Week 4	201.15	78.6	63.8	0.628	0.04
Week 6	203.4	85.7	63.8	0.654	0.01

*[1] Highest combined specificity (Sp) and sensitivity (Se), μmol/L; [2] **Se** = epidemiologic sensitivity; [3] **Sp** = epidemiologic specificity; [4] **AUC** = area under the curve*

very interesting because, in contrast to NEFA, ketones can be simply measured in the field [14].

The optimal threshold of ≥ 600 µmol/L was somewhat lower than the threshold of 800 µmol/L associated with an increased risk of displaced abomasum in Chapinal et al.[15].

Walsh et al.[7] determined BHBA concentration thresholds for the prediction of probability of pregnancy after the first insemination early in lactation. They showed that cows with serum BHBA 1000 µmol/L in the first week or 1400 µmol/L in the second week were significantly less probably to be diagnosed pregnant after first insemination.

The same association between fertility and BHBA concentration has been reported by Ospina et al.[8]. In their study, in animals which were sampled postpartum, the risk of pregnancy within 70 days post-voluntary waiting period (VWP) was reduced by 13% when BHBA concentrations were 970 µmol/L.

In our study, mean BHBA concentration in all sampling times were lower than threshold values reported by Walsh et al.[7] and Ospina et al.[6] that above this, risk of pregnancy after first service is reduced. This fact can point out why BHBA did not affect the probability of pregnancy in this study. In agreement with our results, Fahey et al.[16], Waters et al.[17] and Falkenberg et al.[18] did not report any significant relationship between peripartum BHBA concentrations and reproductive parameters. Moreover, detrimental effects of ketonebodies on reproductive success rely on the longevity of their increased levels [19]. In this study, the duration of increasing BHBA concentration may be too short to have a negative effect on fertility. Therefore, under the conditions of the present study, this variate is not likely to be useful predictors of reproductive performance.

In the current study, higher NEFA concentrations in weeks 4 and 6 postcalving were associated with decreased odds of pregnancy which showed that a moderate degree of fat mobilization in these times of lactation may be critical to get low performance. This shows that postpartum NEFA concentration is a dependable indicator for defining conception status after the first service.

The optimal thresholds in these two times were 201.15 µmol/L and 203.4 µmol/L, respectively. Excessive magnitude or rate of mobilization of fat supplies will cause suboptimal metabolic performance and is likely an indicator of a reduced adaptive response to NEB.

A number of studies have focused on the relationship between NEFA concentrations and reproductive performance [20-22]. The negative impact of NEFA concentration on commencement of luteal activity postpartum was reported during the 4 and 7 week of lactation [21]. A delay in the resumption of ovulation limits the number of oestrous cycles before service, which may lead to the reduced conception rates [23]. However, Reist et al.[24] and Fahey et al.[16] did not report any significant relationships between NEFA concentration and fertility.

After considering the stage of sampling in which significant relationships were observed, we note that these relationships were found in the last weeks of sampling, when the most animals were already cyclic. This is completely reasonable because this is the time that the most of the cows were inseminated for the first time and energy balance indicators are expected to be more informative in this period.

An association between reproductive efficiency in early lactation and elevated peripartum NEFA was reported by Ospina [8]. In all animals sampled prepartum, the risk of pregnancy within 70 d post-VWP was reduced by 19% when NEFA concentrations were ≥270 µmol/L. In all animals sampled postpartum, those with NEFA concentrations ≥720 µmol/L had a 16% decrease in risk of pregnancy.

On the other hand, these thresholds are related to the assessment of conception within 70 days post-voluntary waiting period (VWP). The level at which elevated NEFA is associated with conception at first service was not evaluated.

Although elevated concentrations of both NEFA and BHBA decline the risk of conception, through direct toxic effect on the follicles with induction of cumulus cells apoptosis, necrosis and follicular development arrest [25,26], however, in our study, NEFA concentration was found to have the stronger relationship with reproductive performance than BHBA. This situation is likely because of the more direct physiological relationship between NEFA concentrations and negative energy balance [27].

The current analysis allowed the opportunity to examine the effect of elevated concentrations of pre- and postpartum NEFA and BHBA on reproduction at the cow level. In summary, elevated serum concentrations of BHB within 1 week before calving and NEFA in weeks 4 and 6 after calving were associated with lower probability of pregnancy at the first AI in the present study.

Establish cow level critical thresholds for serum concentrations of NEFA and BHBA to predict conception at first service was a notable feature of the current study. The following cow-level critical thresholds should be considered general guidelines for monitoring cattle: NEFA concentrations ≥201.15 µmol/L for cattle in week 4 postpartum; and NEFA concentrations ≥203.4 µmol/L for cattle in week 6 postpartum. Both postpartum NEFA concentrations above these critical thresholds were associated with decreased occurrence for conception at first service.

This information allows the identification of individual cows at risk for this downstream outcomes based on their NEB status during the transition period. Recognizing cows at risk for decreased PR based on the effects of increased NEFA or BHBA concentrations during the transition period

may help farmers focus on improving herd energy balance. Thus, it is necessary to improvement in management of transition cows so as to minimize the effect of NEB.

Efforts to improve NEB status should be implemented at the herd level, where decisions about nutritional management, diet, comfort, social adaptation, and access to feed, which may be the best methods of minimizing the lagging DMI during the transition period, which is one of the major factors associated with NEB.

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