DOI: 10.1002/vms3.1143

ORIGINAL ARTICLE

WILEY

Association of precalving serum NEFA concentrations with postpartum diseases and reproductive performance in multiparous Holstein cows: Cut-off values

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Funding information
Ferdowsi University of Mashhad, Grant/Award Number: 3/45101

Abstract

Background: High concentrations of NEFA relative to a defined reference or 'cut-point' values before calving can predict the risk of specific or collective periparturient disease events.

Objectives: A field-based cohort study was conducted to evaluate the value and critical points of serum nonesterified fatty acids (NEFA) at the precalving time to predict the occurrence of postpartum diseases and reproductive performance in dairy cows.

Methods: Blood samples were taken from 521 high-yielding dairy cows at 1 week (±3 days) before calving and NEFA levels were measured. Health and reproduction information of each cow includes dystocia, retained placenta, milk fever, metritis, mastitis, pregnancy in the first insemination and pregnancy in the first two inseminations, and culling in the first 60 days of lactation and milk production.

Results: Our results show that there are significant relationships between precalving NEFA with the probability of pregnancy at the first and the first two inseminations after calving. The cows that had NEFA concentrations less than 0.5 mmol/L at the last week of pregnancy were 3.51 and 3.15 times more likely to be pregnant at first insemination and the first two inseminations, respectively. Also, our results showed that there are significant relationships between precalving NEFA concentration and the likelihood of dystocia and milk fever. The probability of dystocia and milk fever occurrence were 2.56 and 1.91 times greater in those cows that had NEFA concentrations more than 0.3 mmol/L, respectively.

Conclusions: The present results indicated that Increasing NEFA during the prepartum period could adversely affect the reproductive efficiency of dairy cows.

KEYWORDS
cut-point, dystocia, fertility, milk fever, NEFA

1 | INTRODUCTION

During the transition period (3 weeks before to 3 weeks after calving) the need for energy increases due to foetal growth, reduced feed intake by cows (between 10% and 30%) and the beginning of the production process (Grummer, 1995). All high-yield dairy cows in this period experience a negative energy balance (Herdt, 2000). The mean duration of negative energy balance during the postpartum period was predicted about 45 days (±21 days) (Herdt, 2000). If a cow is unable to cope with the pregnancy and milk production energy needs, she will develop subclinical and clinical metabolic diseases and might be affected by various infections (Drackley, 1999; Overton & Waldron, 2004). The metabolites nonesterified fatty acids (NEFA) and/or β- hydroxybutyrate (BHB) are common measures of negative energy balance in transition animals (Duffield et al., 2009). The increase in NEFA concentration usually starts about 5 days before calving (LeBlanc et al., 2005). Although some elevation of these metabolites is normal, excessive elevation can indicate poor adaptation to negative energy balance (Herdt, 2000). High concentrations of NEFA relative to a defined reference or 'cut-point' values before calving can predict the risk of specific or collective periparturient disease events (Seifi et al., 2011; Van Saun, 2009). Cows with NEFA and BHBA concentrations in blood above a critical threshold can be predicted to have a greater incidence of diseases such as ketosis and displaced abomasum (LeBlanc et al., 2005; Ospina et al., 2010c; Seifi et al., 2011) and reduced postpartum reproductive performance (Ospina et al., 2010b,c). The objective of the present study was to identify if prepartum NEFA can be used to predict an increased risk of postpartum diseases and reproductive performance in dairy cows and to determine the best cut-points.

2 | MATERIAL AND METHODS

2.1 | Animals and setting

Multiparous Holstein cows from a commercial dairy herd were enrolled as a part of a larger study from November 2017 to October 2019. The dairy herd, a mixed forestall and open shed facility with a TMR feeding system milked 1000 dairy cows three times daily with an average 305-day mature-equivalent milk yield of 11,600 kg. Cows were fed a TMR that was formulated for late pregnant, nonlactating cows (dry cow diet). After calving, cows were fed a lactating cow TMR. The TMR was formulated to meet or exceed NRC (2001) recommendations (Table 1). Feed was provided twice daily.

Cows were scored for body condition on a scale of 1 to 5, in increments of 0.25 (Edmonson et al., 1989), at enrolment (1 = very thin 5 = very obese). All scorings were performed by a single evaluator. Definitions of diseases were as follows: metritis was an enlarged nonpregnant uterus and a watery red-brown fluid to viscous off white purulent cervical or vaginal discharge; retained foetal membranes (RFM) was the retention of foetal membranes for more than 24 h after calving; clinical mastitis was a case of visually abnormal milk associated with hardness and swelling of the affected quarter; lameness was a clinical case of lameness of feet or legs requiring treatment (Loeffler et al., 1999) and locomotion score (LCS) was cauterised 1–5 (score 1 = no lame and score 5 = severe lame) (Sprecher et al., 1997) milk fever was diagnosed as any cow that within 72 h after parturition presented inappetence, nervous symptoms, staggering, varying degrees of unconsciousness, probable sternal recumbency and good response to intravenous calcium treatment; displacement of the abomasum was defined as decreased milk yield accompanied by an audible

TABLE 1 Composition and chemical analysis of close-up and fresh-cow diets.

	Close up	Fresh cows
Ingredient (%; DM basis)		
Concentrate%	21.89	29.68
Oat meal	17.5	16.73
Corn meal	34.5	31.1
Rapeseed meal	13	5.9
Mycofix plus	0.3	0.17
Soybean meal	10	21.7
Fish meal	3.5	3.86
Fat meal	0	1.16
Sodium bicarbonate	0	1.39
Magnesium oxide	0	0.3
Full fat soybean	3	6.78
Calcium carbonate	2	1.38
Salt	0	0.48
Vitamin supplement	1.5	0.78
Mineral supplement	1.5	0.78
Zarin binder (aluminium silicate)	0	0.5
Dicalcium phosphate	0	0.5
PH max	0	0.69
Transitional supplement	10	5.8
Anionic salt (magnesium sulphate)	3.2	0
Alfa-Alfa hay%	5.05	3.92
Corn silage%	60.61	44.51
Wheat straw%	4.04	1.07
Water%	8.41	0
Sugar beet pulp %	0	12.46
Sugar beet molasses %	0	7.11
Cotton seed%	0	1.25
Chemical analysis (%; DM basis)		
DMI	13.5	20.41
NE_L	1.5 mcal/kg	1.61 mcal/kg
CP	14.7	16.7
RUP	35.7	36.1
RDP	19.7	19.2
NDF	28.6	29.1
NFC	38.5	37.5
EE	3.6	4.3
Ca	0.97	0.86
P	0.37	0.48

Zarin binder: toxin binder (processed aluminium silicate).
Transitional supplement: vitamin A 1,500,000 IU/kg, vitamin D3 400,000 IU/kg, vitamin E 20,000 IU/kg, biotin 200 ppm/kg, monensin 3000 ppm/kg.
PH max: a brand of blend that modulate rumen PH.

high-pitched tympanic resonance by simultaneous percussion and auscultation of the left or right abdominal wall between the 9th and 12th rib spaces as diagnosed by the veterinarian (Melendez et al., 2003). Disease diagnoses and pregnancy checks and other disease diagnoses were recorded by the veterinarian of the farm. These diseases were entered on a daily basis into a computerised database. For all diseases other than mastitis, a second occurrence of the disease had to be separated from a prior occurrence by more than 21 days to be counted as a new case. Reproductive management consisted of a voluntary waiting period of 50 days until the first insemination. Thereafter, cows were inseminated following oestrus detection. Pregnancy was diagnosed by transrectal sonography of the uterus at 28–35 days after artificial insemination (AI) and was confirmed at 57–63 days after AI. Pregnancy in the first insemination was the proportion of cows enrolled in the trial that became pregnant after the first insemination and pregnancy in the first two inseminations after calving was the proportion of cows enrolled in the trial that became pregnant after the first or second inseminations.

2.2 | Sample collection and laboratory analyses

A study was conducted to evaluate the association of high serum NEFA concentration shortly before calving with disease occurrence and reproductive outcomes. Blood samples were taken from 521 multiparous Holstein cows 1 week before predicted calving (±3 days).

Blood was collected via the coccygeal vein into 10-mL evacuated tubes with a clot activator. The utmost care was taken to minimise stress during sample collection. The blood tubes are placed diagonally away from direct sunlight for 15 min until the clot is completely formed. Then the samples were centrifuged at 3000 × *g* for 15 min. Serum was harvested immediately and frozen at −20°C until further analysis. For laboratory analysis, after serum thawing, NEFA was measured by BT-1500 auto-analyser (Biotecnica, Rome, Italy), using a commercially available kit (Randox, Ardmore, UK). The intraassay and interassay coefficients of variation were 4.81% and 4.32%, respectively.

2.3 | Statistical analyses

Statistical analyses were performed with SAS software (version 9.4). Initial screening for simple associations of NEFA, at various cut-points, with diseases, culling risk and pregnancy chance were done with contingency tables and chi-square statistics (PROC FREQ in SAS). Cows that had a BCS of 3 were classified as thin, a BCS of 3.25 or 3.5 as fair, and a BCS of more than 3.75 as fat. Parity was classified into two groups: group 1: parities 2 and 3 and group 2: parities 4 and more. The determinants of risk of periparturient diseases, culling and the chance of pregnancy were modeled using multivariable logistic regression (PROC GENMOD with binary estimating distribution, logit link function, and compound symmetry covariance structure). Each model contained the effects of the parity group, BCS category, season and the occurrence of dystocia, retained placenta, metritis, lameness and lameness score, and mastitis. Appropriate cut-points for serum NEFA levels associated with increased risk of cow health events were determined first by creating incremental cut-points of 0.1 mmol/L of NEFA from 0.1 to 1.0 mmol/L. These cut-points were evaluated using dichotomous variables, designating a 0 value for all samples below each cut-point and assigning a value of 1 to all values at or above each cut-point. These serial thresholds were then contrasted with the occurrence of clinical disease using simple 2 × 2 contingency tables. For metabolites that were retained in the final models, animals were classified as being above or below a series of cut-points, each of which was tested for association with subsequent outcomes. To determine the best cut-point, the sensitivity and specificity of the cut-points were calculated manually and then the cut-point that had the highest sum of sensitivity and specificity was considered the optimal cut-point.

3 | RESULTS

Our results indicated that there were significant relationships between precalving NEFA concentrations with the occurrence of dystocia and milk fever and the probability of pregnancy in the first insemination (Preg1) and the first two inseminations (Preg2) (Table 2). In the present study, no significant relationship was found between precalving NEFA with RFM, metritis, mastitis, displacement of the abomasum (DA) and culling in the first 60 days of lactation.

There was a significant relationship between NEFA concentrations with milk fever occurrence ($p = 0.0348$) on account of intervening factors such as parity and season (Tables 2 and 3). The cows which had serum NEFA concentrations greater than 0.3 mmol/L 1 week before calving was 1.91 times at greater risk of milk fever. The sensitivity and specificity for the proposed cut-point were 27.16% and 86.82%, respectively (Table 2). The results suggested that there was a significant relationship between precalving NEFA concentration with dystocia occurrence ($p = 0.0081$). Our study proposed a cut-point of 0.3 mmol/L to predict dystocia occurrence (Tables 2 and 4). Accounting for the factors such as parity, season, locomotion score, and body condition score, those cows that had blood NEFA concentrations greater than 0.3 mmol/L were 2.56 times more likely to develop dystocia (Tables 2 and 4).

Accounting for the effects of parity, BCS, locomotion score, milk fever, dystocia, RFM, metritis, mastitis and abomasum displacement, 1 week before calving NEFA concentration was associated with the likelihood of pregnancy in the first insemination after calving. Cows that had serum NEFA concentrations less than 0.5 mmol/L 1 week before calving were 3.51 times more likely to become pregnant (Tables 2 and 5). In addition, our results indicated that there was a significant relationship between precalving NEFA concentration with pregnancy in the first two inseminations ($p = 0.0098$), accounting for the effects of parity, locomotion score, body condition score, parturition season, milk fever, abomasum displacement, retained placenta, dystocia, metritis and mastitis. The probability of pregnancy in the first two postpartum inseminations was 3.15 times greater in those cows that had NEFA concentrations less than 0.5 mmol/ L before calving (Tables 2 and 6).

TABLE 2 Association between serum NEFA concentrations at 1 week before calving with postpartum reproductive and health events.

	Cut-point	Proportion of animals at or above	Odds ratio	95% CI	p Value	Sen%	Sp%
Milk fever	>0.3	15.36	1.91	1.05–3.5	0.0348	27.16	86.82
Dystocia	>0.3*	15.36	2.56	1.27–5.13	0.0081	29.55	85.95
	>0.4	9.4	2.8	1.26–6.25	0.0115	20.45	91.61
	>0.5	6.14	2.73	1.06–7.06	0.0371	13.64	94.55
	>0.6	3.84	3.94	1.36–11.43	0.0114	11.36	96.86
Preg1	<0.3	15.36	3.55	1.71–7.34	0.007	6.04	80.91
	<0.4	9.4	2.47	1–6.01	0.0486	4.03	88.44
	<0.5*	6.14	3.51	1.03–11.94	0.0438	2.01	92.2
Preg2	<0.2	29.75	1.79	1.2–2.66	0.0039	22.75	63.53
	<0.3	15.36	2.27	1.33–3.88	0.0027	9.02	78.57
	<0.4	9.4	2.06	1.06–4	0.0328	5.49	86.84
	<0.5*	6.14	3.15	1.32–7.56	0.0098	2.75	90.6

The models account for the significant effects of parity, body condition score, Locomotion Score (LCS), dystocia, milk fever, metritis, mastitis, displacement of abomasum and retained placenta on occurrence of reproductive and health events.
*Cut-off point with greatest sum of sensitivity and specificity.
Preg2, pregnancy in first two inseminations; Preg1, pregnancy in first insemination; DA, displacement of abomasum.

TABLE 3 Final analysis of multivariate logistic regression (GENMOD) on the relationship between NEFA concentration with milk fever by considering intervention factors such as season, parity, body condition score (BCS), locomotion score (LCS) and dystocia.

Parameter	Odds ratio	95% CI		p Value
Intercept				0.3711
NEFA <0.3 mmol/L	1.91	1.04	3.5	0.0348
NEFA >0.3 mmol/L	Referent			
Parity <3	3.01	1.79	5.07	<0.0001
Parity >4	Referent			
Season: spring	2.21	1.04	4.7	0.0391
Season: summer	1.44	1.33	2.77	0.2765
Season: autumn	1.55	1.27	2.52	0.4884
Season: winter	Referent			

TABLE 4 Final logistic regression model of the association between NEFA concentrations in prepartum with dystocia, adjusted for the effects of season, parity, body condition score (BCS) and locomotion score (LCS).

Parameter	Odds ratio	95% CI		p Value
Intercept				<0.0001
NEFA <0.3 mmol/L	2.56	1.27	5.15	0.0081
NEFA >0.3 mmol/L	referent			

TABLE 5 Final logistic regression model of the association between NEFA concentrations in prepartum with the probability of pregnancy in first insemination (preg1), adjusted for the effects of parity, season, body condition score (BCS), locomotion score (LCS), dystocia, milk fever, retained placenta (RFM), metritis, displacement of abomasum (LDA) and mastitis.

Parameter	Odds ratio	95% CI		p Value
Intercept				<0.0001
NEFA <0.5 mmol/L	3.51	1.03	11.94	0.0438
NEFA >0.5 mmol/L	Referent			
Parity <3	1.9	1.25	2.87	0.0025
Parity >4	Referent			
Season: spring	1.33	1.3	2.23	0.3527
Season: summer	2.23	1.22	3.79	0.0081
Season: autumn	1.66	1.03	1.8	0.9003
Season: winter	Referent			

4 | DISCUSSION

This study has found that prepartum elevation of serum NEFA is associated with the occurrence of dystocia, milk fever and a lower probability of pregnancy. Our results indicated that there was a significant relationship between NEFA concentrations 1 week before calving with the probability of pregnancy at first insemination after calving. Cows with NEFA concentrations less than 0.5 mmol/L were 3.5 times more likely to become pregnant at first insemination. Furthermore, we found a significant relationship between NEFA concentrations at 1 week before calving with the probability of pregnancy in the first two inseminations. The odds of pregnancy at the first two inseminations were 3.1 times greater in cows with serum NEFA concentrations of less than 0.5 mmol/L in the first week before calving.

The cut-off point proposed by our study is higher than the cut-off point presented by other studies (0.27 and 0.4 mmol/L)

TABLE 6 Final logistic regression model of the association between NEFA concentrations in prepartum with the probability of pregnancy in the sum of first two insemination (preg2), adjusted for the effects of parity, season, body condition score (BCS), locomotion score (LCS), dystocia, milk fever, retained placenta (RFM), metritis, displacement of abomasum (LDA) and mastitis.

Parameter	Odds ratio	95% CI		*p* Value
Intercept				<0.0001
NEFA <0.5 mmol/L	3.15	1.31	7.56	0.0098
NEFA >0.5 mmol/L	Referent			
Parity <3	2.03	1.41	2.92	0.0001
Parity >4	Referent			
No RFM	1.99	1.14	3.47	0.0148
Yes RFM	Referent			

(McArt et al., 2013; Melendez et al., 2009; Ospina et al., 2010a); however, researchers recently have used a similar cut-point to detect negative energy balance in UK dairy farms (Macrae et al., 2019). There are limited studies to predict the chance of pregnancy at the first and the first two inseminations after calving by providing a cut-off point for NEFA 1 week before calving. McArt et al. (2013) reported that following the increase of NEFA concentration in the precalving period to more than 0.3 mmol/L, the probability of pregnancy in the first postpartum insemination decreases. Contrary to our findings, Chapinal et al. (2012), who sampled cows weekly from 1 week before to 3 weeks after calving, found no association between NEFA concentrations with the likelihood of pregnancy at the first postpartum insemination. In addition, Garverick et al. (2013) reported that NEFA concentration was not significantly different between pregnant and nonpregnant cows (from 3 weeks before calving to the third day after calving) after the first postpartum insemination. Rodríguez et al. (2020) also reported that there is no relationship between NEFA concentrations with fertility. Ospina et al. (2010a,b,c) conducted a large-scale study to provide cut-off points for NEFA and BHBA around calving. They proposed a cut-point of 0.3 and 0.6 mmol/L for at 1 week before and after calving, respectively. They believed that the likelihood of occurrence of diseases in cows that had NEFA more than the proposed cut-point was higher than in cows that NEFA lower than this cut-point (Ospina et al., 2010). Furthermore, it has been reported that if more than 15% of herds have energy-related metabolites higher than the mentioned values not only increases the probability of disease in that herd but reproductive and production efficiencies would be affected (Wankhade et al., 2017). Potential mechanisms for these detrimental effects on reproductive performance might include induction of apoptosis in granulosa cells, inhibition of follicle growth and delay in the onset of ovulation (Butler et al., 2006; Friggens, 2003; Jorritsma et al., 2004; Leroy et al., 2006; Souissi & Bouraoui, 2019).

We found that there was a significant relationship between NEFA concentrations at 1 week before calving with the probability of occurrence of milk fever. Cows with NEFA concentrations greater than 0.3 mmol/L were 1.9 times more likely to develop milk fever. Neves et al. (2017) reported that there was no association between increased concentrations of blood energy-related metabolites (NEFA and BHBA) in the prepartum and the occurrence of hypocalcaemia at the time of calving. On the other hand, Melendez et al. (2009) indicated that cows with NEFA concentrations of more than 1.2 mmol/L had a higher chance of occurrence of milk fever and mastitis. It is difficult to explain the relationship between the increase in NEFA concentration and milk fever because they have no direct causal relationship. However, decreasing dry matter intake and increasing BHBA and NEFA concentration in cows around calving can lead to an increased likelihood of hypocalcaemia and ketosis (Chapinal et al., 2011; LeBlanc et al., 2005; Martinez et al., 2012).

There was a significant relationship between precalving NEFA concentration and with the likelihood of dystocia occurrence in this study. The odds of occurrence of dystocia were 2.6 times greater in cows with serum NEFA concentrations higher than 0.3 mmol/L in 1 week before calving. In previous studies, high concentrations of NEFA before parturition have been mentioned as a risk factor for the occurrence of dystocia, retained placenta, ketosis, abomasum displacement and mastitis (Chapinal et al., 2011; Dyk, 1995; Kaneene et al., 1997; Moyes et al., 2009). It was mentioned that improving the ratio of energy and protein in transition cows can prevent the occurrence of such diseases and disorders (Duffield et al., 1999; Duffield et al., 2002; Grummer, 1995; Kaneene et al., 1997; Oetzel, 2004; Vandehaar et al., 1999).

5 | CONCLUSION

The findings of this study suggested that increased NEFA concentration in prepartum can lead to decreasing chance of pregnancy. We suggest that 0.5 mmol/L is a better cut-points to predict sound fertility. Furthermore, our result reinforces the predictive association of elevated concentrations of NEFA in a close-up period with the risk of dystocia and milk fever at the cut-points of 0.3 mmol/L. Thus, measurement of NEFA concentrations in the week before calving may provide useful supplementary information for herd health and reproduction monitoring.

AUTHOR CONTRIBUTIONS

Samuel Kia: investigation; data curation; validation; writing original draft. **Hesam A Seifi**: conceptualisation; data curation; formal analysis; funding acquisition; methodology; project administration; resources; supervision; validation; writing-review & editing. **Mehrdad Mohri**: supervision; validation; methodology; project administration.

ACKNOWLEDGEMENTS

The authors would like to thank the Research Deputy of Ferdowsi University of Mashhad for the financial support of this study.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received (45101). The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

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PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/vms3.1143.

REFERENCES

Butler, S. T., Pelton, S. H., & Butler, W. R. (2006). Energy balance, metabolic status, and the first postpartum ovarian follicle wave in cows administered propylene glycol. *Journal of Dairy Science*, *89*(8), 2938–2951.

Chapinal, N., Carson, M., Duffield, T. F., Capel, M., Godden, S., Overton, M., Santos, J. E., & LeBlanc, S. J. (2011). The association of serum metabolites with clinical disease during the transition period. *Journal of Dairy Science*, *94*(10), 4897–4903.

Chapinal, N., Carson, M. E., LeBlanc, S. J., Leslie, K. E., Godden, S., Capel, M., Santos, J. E., Overton, M. W., & Duffield, T. F. (2012). The association of serum metabolites in the transition period with milk production and early-lactation reproductive performance. *Journal of Dairy Science*, *95*(3), 1301–1309.

Drackley, J. K. (1999). Biology of dairy cows during the transition period: The final frontier? *Journal of dairy science*, *82*(11), 2259–2273.

Duffield, T., Bagg, R., DesCoteaux, L., Bouchard, E., Brodeur, M., DuTremblay, D., Keefe, G., LeBlanc, S., & Dick, P. (2002). Prepartum monensin for the reduction of energy associated disease in postpartum dairy cows. *Journal of Dairy Science*, *85*(2), 397–405.

Duffield, T. F., Leslie, K. E., Sandals, D., Lissemore, K., McBride, B. W., Lumsden, J. H., Dick, P., & Bagg, R. (1999). Effect of a monensin-controlled release capsule on cow health and reproductive performance. *Journal of Dairy Science*, *82*(11), 2377–2384.

Duffield, T. F., Lissemore, K. D., McBride, B. W., & Leslie, K. E. (2009). Impact of hyperketonemia in early lactation dairy cows on health and production. *Journal of Dairy Science*, *92*(2), 571–580.

Dyk, P. B. (1995). Prepartum non-esterified fatty acids in plasma are higher in cows developing periparturient health problems. *Journal of Dairy Science*, *78*, 264.

Edmonson, A. J., Lean, I. J., Weaver, L. D., Farver, T., & Webster, G. (1989). A body condition scoring chart for Holstein dairy cows. *Journal of Dairy Science*, *72*(1), 68–78.

Friggens, N. C. (2003). Body lipid reserves and the reproductive cycle: Towards a better understanding. *Livestock Production Science*, *83*(2-3), 219–236.

Garverick, H. A., Harris, M. N., Vogel-Bluel, R., Sampson, J. D., Bader, J., Lamberson, W. R., Spain, J. N., Lucy, M. C., & Youngquist, R. S. (2013). Concentrations of nonesterified fatty acids and glucose in blood of periparturient dairy cows are indicative of pregnancy success at first insemination. *Journal of Dairy Science*, *96*(1), 181–188.

Grummer, R. R. (1995). Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *Journal of Animal Science*, *73*(9), 2820–2833.

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

Jorritsma, R., Cesar, M. L., Hermans, J. T., Kruitwagen, C. L., Vos, P. L., & Kruip, T. A. (2004). Effects of non-esterified fatty acids on bovine granulosa cells and developmental potential of oocytes in vitro. *Animal Reproduction Science*, *81*(3-4), 225–235.

Kaneene, J. B., Miller, R., Herdt, T. H., & Gardiner, J. C. (1997). The association of serum nonesterified fatty acids and cholesterol, management and feeding practices with peripartum disease in dairy cows. *Preventive Veterinary Medicine*, *31*(1-2), 59–72.

LeBlanc, S. J., Leslie, K. E., & Duffield, T. F. (2005). Metabolic predictors of displaced abomasum in dairy cattle. *Journal of Dairy Science*, *88*(1), 159–170.

Leroy, J. L., Vanholder, T., Opsomer, G., Van Soom, A., & de Kruif, A. (2006). The in vitro development of bovine oocytes after maturation in glucose and β-hydroxybutyrate concentrations associated with negative energy balance in dairy cows. *Reproduction in Domestic Animals*, *41*(2), 119–123.

Loeffler, S. H., de Vries, M. J., & Schukken, Y. H. (1999). The effects of time of disease occurrence, milk yield, and body condition on fertility of dairy cows. *Journal of Dairy Science*, *82*(12), 2589–2604.

Macrae, A. I., Burrough, E., Forrest, J., Corbishley, A., Russell, G., & Shaw, D. J. (2019). Prevalence of excessive negative energy balance in commercial United Kingdom dairy herds. *The Veterinary Journal*, *248*, 51–57.

Martinez, N., Risco, C. A., Lima, F. S., Bisinotto, R. S., Greco, L. F., Ribeiro, E. S., Maunsell, F., Galvão, K., & Santos, J. E. (2012). Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. *Journal of Dairy Science*, *95*(12), 7158–7172.

McArt, J. A., Nydam, D. V., Oetzel, G. R., Overton, T. R., & Ospina, P. A. (2013). Elevated non-esterified fatty acids and β-hydroxybutyrate and their association with transition dairy cow performance. *The Veterinary Journal*, *198*(3), 560–570.

Melendez, P., Donovan, G. A., Risco, C. A., Littell, R., & Goff, J. P. (2003). Effect of calcium-energy supplements on calving-related disorders, fertility and milk yield during the transition period in cows fed anionic diets. *Theriogenology*, *60*(5), 843–854.

Melendez, P., Marin, M. P., Robles, J., Rios, C., Duchens, M., & Archbald, L. (2009). Relationship between serum nonesterified fatty acids at calving and the incidence of periparturient diseases in Holstein dairy cows. *Theriogenology*, *72*(6), 826–833.

Moyes, K. M., Larsen, T., Friggens, N. C., Drackley, J. K., & Ingvartsen, K. L. (2009). Identification of potential markers in blood for the development of subclinical and clinical mastitis in dairy cattle at parturition and during early lactation. *Journal of Dairy Science*, *92*(11), 5419–5428.

Neves, R. C., Leno, B. M., Stokol, T., Overton, T. R., & McArt, J. A. (2017). Risk factors associated with postpartum subclinical hypocalcemia in dairy cows. *Journal of Dairy Science*, *100*(5), 3796–3804.

Oetzel, G. R. (2004). Monitoring and testing dairy herds for metabolic disease. *Veterinary Clinics: Food Animal Practice*, *20*(3), 651–674.

Ospina, P. A., Nydam, D. V., Stokol, T., & Overton, T. R. (2010a). Associations of elevated nonesterified fatty acids and β-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. *Journal of Dairy Science*, *93*(4), 1596–1603.

Ospina, P. A., Nydam, D. V., Stokol, T., & Overton, T. R. (2010b). Evaluation of nonesterified fatty acids and β-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. *Journal of Dairy Science*, *93*(2), 546–554.

Ospina, P. A., Nydam, D. V., Stokol, T., & Overton, T. R. (2010c). Association between the proportion of sampled transition cows with increased nonesterified fatty acids and β-hydroxybutyrate and disease incidence, pregnancy rate, and milk production at the herd level. *Journal of Dairy Science*, *93*(8), 3595–3601.

Overton, T. R., & Waldron, M. R. (2004). Nutritional management of transition dairy cows: Strategies to optimize metabolic health. *Journal of Dairy Science*, *87*, E105–e119.

Rodríguez, A., Mellado, R., & Bustamante, H. (2020). Prepartum fat mobilization in dairy cows with equal body condition and its impact on health, behavior, milk production and fertility during lactation. *Animals*, *10*(9), 1478.

Seifi, H. A., LeBlanc, S. J., Leslie, K. E., & Duffield, T. F. (2011). Metabolic predictors of post-partum disease and culling risk in dairy cattle. *The Veterinary Journal*, *188*(2), 216–220.

Souissi, W., & Bouraoui, R. (2019). Relationship between body condition score, milk yield, reproduction, and biochemical parameters in dairy cows. In N. M'Hamdi (Ed.), Lactation in farm animals – Biology, physiological basis, nutritional requirements, and modelization. IntechOpen.

Sprecher, D. E., Hostetler, D. E., & Kaneene, J. B. (1997). A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. *Theriogenology*, *47*(6), 1179–1187.

Vandehaar, M. J., Yousif, G., Sharma, B. K., Herdt, T. H., Emery, R. S., Allen, M. S., & Liesman, J. S. (1999). Effect of energy and protein density of prepartum diets on fat and protein metabolism of dairy cattle in the periparturient period. *Journal of Dairy Science*, *82*(6), 1282–1295.

Van Saun, R. J. (2009). Metabolic profiling. In *Current veterinary therapy* (pp. 153–162). Elsevier Inc.

Wankhade, P. R., Manimaran, A., Kumaresan, A., Jeyakumar, S., Ramesha, K. P., Sejian, V., Rajendran, D., & Varghese, M. R. (2017). Metabolic and immunological changes in transition dairy cows: A review. *Veterinary World*, *10*(11), 1367.

How to cite this article: Kia, S., Mohri, M., & Seifi, H. A. (2023). Association of precalving serum NEFA concentrations with postpartum diseases and reproductive performance in multiparous Holstein cows: Cut-off values. *Veterinary Medicine and Science*, 1–7. https://doi.org/10.1002/vms3.1143