Using Physiological Markers to Detect Health and Production Problems in Transition Dairy Cows

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Take Home Messages

- High concentrations of blood metabolites associated with negative energy balance (e.g. NEFA and BHBA) around calving have been used to predict cows at risk for disease, low milk yield and poor reproductive performance.
- New research shows that cows with high concentrations of stress or inflammation biomarkers around calving are also at increased risk for health and production problems.
- Cows with high fecal cortisol metabolite concentrations before calving, a measure of physiological stress, were more likely to get sick after calving and less likely to become pregnant by 150 DIM. Higher fecal cortisol metabolite concentrations after calving were also associated with lower 305-day projected milk yield in multiparous cows.
- Inflammation associated with infection or injury increases haptoglobin (Hp) concentrations. High Hp concentrations during the week before or after calving were associated with a decreased risk of pregnancy in primiparous cows. Projected 305-day milk yield was lower for multiparous cows with high Hp during the week before calving and projected milk yields were lower for all cattle with high Hp during the week after calving.
- Finding better ways to detect animals that are at risk for health or production problems will help with early treatment or preventative management strategies that may prevent these problems from progressing to their clinical and consequently most costly stage.

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Introduction

The use of blood metabolites for herd-level health assessment during the period around calving (transition period) has been an area of study for many years (Ingraham and Kappel, 1988; Oetzel, 2004). There is renewed interest in this topic, however, as new research shows that blood metabolite concentrations around calving not only can predict disease, but also are associated with economically important herd parameters including milk yield and reproductive performance. Much of this work has focused on metabolites related to negative energy balance including nonesterified fatty acid (**NEFA**) and beta-hydroxybutyrate (**BHBA**); however, biomarkers of stress and inflammation around calving may also be useful for assessing transition cow health and performance.

This paper reviews current knowledge of physiological biomarkers that can be used to predict disease, low milk yield and compromised reproductive performance.

Biomarkers of Negative Energy Balance – NEFA & BHBA

Nearly all cows undergo a period of negative energy balance after calving as dry matter intake is often insufficient to meet the increased energy demands of lactation (Grummer et al., 2004). Cows with the greatest increases in NEFA and BHBA concentrations around calving, reflective of more severe negative energy balance, are most likely to develop postpartum disease, have low milk yield and poor reproductive performance.

In a series of studies conducted at Cornell University, Ospina et al. (2010a, 2010b) described the associations of pre- and postpartum NEFA and BHBA concentrations with postpartum health, milk production and reproductive performance. To be included in these studies herds must have: 1) had more than 250 milking cows, 2) housed cows in free-stalls, 3) fed a total mixed ration (TMR), and 4) participated in DHIA or used Dairy Comp 305 (Valley Ag. Software, Tulare, CA). On each farm 15 cows were randomly selected from a prepartum group (14 to 2 days prepartum) and 25 cows were selected from a postpartum group (3 to 14 days postpartum). Blood was collected from the coccygeal (tail) vein or artery into a red-top serum tube. The serum collected was then analyzed for NEFA and BHBA concentration. For each experimental animal, herd records were used to identify cases of displaced abomasum (**DA**), clinical ketosis (**CK**), metritis and/or retained placenta (**MET**) within 30 days in milk, time to pregnancy within 70 days post voluntary waiting period, and projected Mature Equivalent 305 day milk yield at 120 days in milk. In total 2758 cows were sampled (1440 animals prepartum and 1318 animals postpartum).

A summary of the critical concentration thresholds identified for NEFA and BHBA that predicted increased disease risk, lower milk yield and compromised reproductive performance are presented in Table 1. There were no parity differences influencing the associations of NEFA and BHBA concentrations with disease or reproductive performance after calving. However, the association between postpartum concentrations of these metabolites and milk yield differed between heifers and cows. Among heifers, higher NEFA and BHBA concentrations during the postpartum period were associated with greater projected milk yield, while in multiparous cows higher concentrations of these metabolites were associated with lower projected milk yield (Table 1). On the other hand, a high concentration of NEFA during the prepartum period was associated with lower predicted milk yield in both heifers and cows (Ospina et al. 2010a, 2010b).

Table 1. Summary of critical NEFA and BHBA thresholds[1] as predictors of disease, milk yield and reproductive performance during the 2 week period before and after calving (Ospina et al. 2010a, 2010b).

Prepartum (14 to 2 d before calving)	
NEFA > 0.29 mEq/L	Cattle 1.6 times more likely to develop DA, CK or MET[2]
NEFA > 0.27 mEq/L	19% decreased risk of conception in all cattle[3]
NEFA > 0.33 mEq/L	683 kg lower mature equivalent 305-d milk yield
Postpartum (3 to 14 d after calving)	
NEFA > 0.57 mEq/L	Cattle 1.9 times more likely to develop DA, CK or MET[2]
	Heifers: 488 kg higher mature equivalent 305-d milk yield
NEFA > 0.72 mEq/L	16% decreased risk of conception in all cattle[3]
	Cows: 647 kg lower ME 305-d milk yield
BHBA > 10 mg/dL	Cattle 3.1 times more likely to develop DA, CK or MET[2]
	13% decreased risk of conception in all cattle[3]
	Cows: 393 kg lower mature equivalent 305-d milk yield
BHBA > 9 mg/dL	*Heifers*: 403 kg higher mature equivalent 305-d milk yield

[1] The statistical analysis to determine these thresholds controlled for body condition score, calving season, milk production and parity.
[2] Displaced abomasum (DA), Clinical Ketosis (CK), Metritis (MET)
[3] Risk of conception within 70-d post voluntary waiting period

These results highlight the importance of maintaining adequate energy intake during the weeks before and after calving to control energy status and minimize risk for disease, lower milk yield and poor reproductive performance. The results of Ospina et al. (2010a, 2010b) are consistent with the results of other researchers who have demonstrated that high NEFA and BHBA concentrations around calving are associated with increased disease risk (Kaneene et al. 1997; Chapinal et al. 2011; Huzzey et al. 2012), lower milk yield (Duffield et al., 2009; Chapinal et al. 2012) and longer days to conception (Walsh et al., 2007).

Although evidence continues to mount in support of NEFA and BHBA as useful metabolites for predicting health status and performance, researchers are interested in exploring whether there are other meaningful physiological markers that can be used to monitor health and performance around calving.

■ Biomarkers of Stress and Inflammation

Stress

The transition period is one of the most stressful periods in a dairy cow's lactation cycle. Not only must she contend with nutritional and physiological changes that must occur for her to begin lactation following calving, but she must also cope with numerous environmental and management related changes, including social regroupings, mixing of heifers and cows, and novel environments like the milk parlor (particularly for heifers). These situations represent potential stressors that may affect health and performance if individual coping strategies cannot effectively adapt to these challenges. Physiological markers of stress, particularly during the period before calving, may reveal opportunities for improvements in transition cow management.

Plasma cortisol has long been used to measure physiological stress in animals; however, there are many challenges with using this marker as a field diagnostic measure. Restraint and handling, which are required during blood sampling, can raise blood cortisol concentrations quickly (Cook et al., 2000). Further, cortisol concentrations in the blood fluctuate greatly across the day due to the normal secretory pattern and are subject to substantial individual variation (Thun et al., 1981). Multiple blood samples would be required to obtain an accurate assessment of stress from plasma cortisol values and this is not practical for herd testing programs aimed at evaluating health.

Fecal cortisol metabolites (11,17-dioxyandrostanes) may be an alternative to plasma cortisol as a measure of the stress response in cattle, since their concentrations are not influenced by handling stress and are less variable over the course of the day (Palme et al., 1999).

Inflammation

The period around calving is also associated with a high degree of inflammation (Bertoni et al., 2008). The calving event is extremely physically demanding and tissue damage and swelling, particularly around the vulva, are common during the delivery process. The days around calving are associated with a period of immune activation and increased concentrations of plasma proteins associated with inflammation (Uchida et al., 1993). These inflammatory markers are elevated at parturition and the days following in all cattle, although these increases appear to be more pronounced in cattle that go on to have more serious health complications (Huzzey et al., 2009).

Over the last decade there has been growing interest in monitoring inflammatory responses in animals for clinical or experimental purposes (Eckersall, 2000). Measures of immune activation (e.g. induction of the acute phase response) during the transition period may provide information about an animal's level of risk for developing subsequent health and production complications. The acute phase protein haptoglobin (**Hp**), a non-specific marker of inflammation, injury, or infection has been shown to be particularly useful for the early identification of common transition cow disorders including metritis (Huzzey et al., 2009) and mastitis (Hirvonen et al., 1996). For example, cows with Hp > 1 g/L on day 3 after calving were nearly 7 times more likely to develop mild or severe metritis. The acute phase response may also be sensitive to environmental stressors (Lomborg et al., 2008) and therefore further investigation into their usefulness for monitoring transition cow health and performance is warranted.

Markers of Stress and Inflammation to Identify High Risk Cows

Researchers at Cornell University conducted a study on 2 commercial dairy farms to determine if fecal cortisol metabolites (**F.CORT**) and Hp, markers of stress and inflammation respectively, could be used to identify animals at increased risk for disease or performance problems. A total of 412 Holstein dairy cattle were enrolled in the study between the two farms. At each dairy blood and fecal samples were collected from cows beginning 3 weeks before calving (wk -3, wk -2 and wk -1) and one sample was collected within 3 to 10 days after calving (Postpartum). Blood samples were used to measure Hp and NEFA concentrations while the fecal sample was used to determine F.CORT (11,17-dioxyandrostane) concentrations.

All experimental animals were followed for 30 DIM to record cases of retained placenta (**RP**), displaced abomasum (**DA**) and death (not including voluntary culls). The postpartum blood sample was used to diagnose subclinical ketosis (**SCK**) when plasma BHBA was ≥ 10mg/dl (Ospina et al., 2010a) and High Haptoglobin (**HiHp**, suggestive of an infection such as metritis) when plasma Hp was > 1.0 g/L (Huzzey et al., 2009). Because our interest was in

identifying biomarkers that could predict disease, only prepartum concentrations of the biomarkers of interest were compared with postpartum health status. The experimental cows were divided into 3 health categories for this analysis: 1) No Disorder of Interest (NDI), 2) One disorder (RP, DA, SCK or HiHp), and 3) More than one disorder (RP, DA, SCK, HiHp) or death.

As expected prepartum plasma NEFA was a strong predictor of postpartum health; however, this relationship depended on parity and the degree of illness after calving. Cows that developed multiple disorders after calving or that died had the greatest concentrations of NEFA relative to the other two health categories, particularly during the 2-week period before calving. These differences in prepartum NEFA concentrations between health categories were more pronounced in multiparous cows than primiparous cows. There were no associations between prepartum Hp or F.CORT concentration and the occurrence of one disorder (RP, DA, SCK or HiHP) by 30 DIM. Hp concentration tended to be 23 to 43% higher during wk -2 and -1 and FCORT tended to be 11% higher during wk -3 and -2 for cows that developed more than one disorder or that died by 30 DIM relative to cows in the NDI category; however, neither of these analytes could predict which cows would go on to develop health complications as well as prepartum NEFA concentration (Huzzey et al., 2011).

To evaluate the relationships of these analytes with subsequent mild yield and reproductive performance, herd Dairy Comp 305 records were used to collect information on each cow's 305-day mature equivalent (305ME) milk yield from the 3rd DHI test day (about 102 days in milk) and number of days to conception. A range of possible concentration thresholds for each biomarker (pre- and postpartum) were evaluated and the effect of being above or below this threshold on predicted 305ME and risk of pregnancy by 150 DIM was evaluated. The threshold that was most strongly associated with changes in milk yield or reproductive performance was selected. Cows that had concentrations of the biomarker above the threshold were considered the high-risk group while cows below the threshold were considered the low-risk group.

Higher prepartum NEFA and Hp concentrations before and after calving were associated with lower projected 305ME milk yield ($P \leq 0.01$) more so among the multiparous cows than the primiparous cows (Table 2). After calving all the biomarkers of interest were associated with 305ME milk yield projections. Similar to the results of Ospina et al. (2010b) primiparous cows with higher NEFA after calving also had higher projected 305ME milk yield ($P = 0.02$) while multiparous cows with higher NEFA after calving tended to have lower projected milk yield ($P = 0.08$; Table 2). Hp > 1.1 g/L after calving was associated with a 947 kg lower projected 305ME milk yield for both multiparous and primiparous cows ($P = 0.001$), while higher F.CORT

concentrations after calving were associated with lower projected 305ME milk yield for multiparous cows only (*P* = 0.03; Table 2).

Table 2. Difference in projected 305-day mature equivalent (305ME) milk yield for cows that were above the analyte concentration threshold compared to those cows that were below the threshold.

Analyte Threshold[1]	At risk group (MP or PP)[2]	Proportion of animals above threshold	Difference in 305ME Milk Yield (kg)[3]	SE of the Difference
Prepartum (Week -2)				
NEFA > 0.45 mEq/L	MP	16 %	- 1464	411
Prepartum (Week -1)				
NEFA > 0.55 mEq/L	MP, PP	19 %	- 1360	394
Hp > 0.20 g/L	MP	23 %	- 943	394
Postpartum (3 to 10 DIM)				
NEFA > 0.70 mEq/L	PP	15 %	+ 1049	428
NEFA > 0.70 mEq/L	MP	25 %	- 592	333
Hp > 1.10 g/L	MP, PP	33 %	- 947	318
F.Cort > 400 ng/g DM	MP	32 %	- 663	308

[1] Each analyte during each period was tested in a separate model which accounted for the following covariates: Farm, BCS, somatic cell count linear score, DIM at test day and NEFA concentration in the Hp and F.CORT models.

[2] Parity group for which the association between the analyte and milk yield was significant (MP = multiparous cows; PP = primiparous cows).

[3] Difference relative to the average 305ME milk yield for cows below the concentration threshold.

Associations between the analytes of interest and reproductive performance were generally limited to primiparous cows. Primiparous cows with F.CORT > 2300 ng/g fecal DM during the 2-week period before calving had a 36 to 42% decreased risk of pregnancy by 150 DIM, suggesting that increased prepartum stress is associated with compromised reproductive performance (Table 3). Primiparous cows with Hp > 0.4 g/L during the week before calving or Hp > 1.3 g/L during the week after calving had a 41% decreased risk of pregnancy by 150 DIM (Table 3). Similar to Ospina et al. (2010b), higher NEFA concentrations during the week before and after calving were associated with a decreased risk of pregnancy (42% and 39%, respectively; Table 3), however in the current study these associations were also limited to primiparous cows.

Table 3. The effects of analyte concentration thresholds on risk of conception by 150 DIM (Cox proportional hazard ratio) at three sample periods relative to calving for 412 Holstein dairy cows (230 multiparous cows and 182 primiparous cows).

Analyte Threshold[1]	At risk group (MP or PP) [2]	Proportion of animals above threshold	Hazard Ratio [3]	*P*
Prepartum (Week -2)				
Hp > 0.70 g/L	MP	9 %	2.02	0.02
F.Cort > 2300 ng/g DM	PP	30 %	0.64	0.05
Prepartum (Week -1)				
NEFA > 0.40 mEq/L	PP	44 %	0.58	0.02
Hp > 0.40 g/L	PP	22 %	0.59	0.05
F.Cort > 2300 ng/g DM	PP	42 %	0.58	0.02
Postpartum (3 to 10 DIM)				
NEFA > 0.45 mEq/L	PP	40 %	0.61	0.02
Hp > 1.30 g/L	PP	31 %	0.59	0.02

[1] Each analyte during each period was tested in a separate model which accounted for the following covariates: Farm, BCS, somatic cell count linear score, DIM at test day and NEFA concentration (except for NEFA models).

[2] Parity group for which the association between the analyte and milk yield was significant (MP = multiparous cows; PP = primiparous cows)

[3] A hazard ratio > 1 indicates a positive relationship with reproductive performance (ie. risk of pregnancy was greater for cows above the indicated cutpoint, while a hazard ration < 1 indicates a negative relationship (risk of pregnancy was lower cows above the indicated cutpoint). For example a HR = 0.59 is interpreted as follows: cows that were above the concentration threshold had a 41% decreased risk of conception by 150 DIM (1 – 0.59) compared to cows below the threshold.

■ Interpretation of Physiological Biomarkers for Herd Health Assessment

As a tool for monitoring herd heath, analyte testing will likely be more practical when considered at the herd rather than the individual level. Recommendations are to sample 12 to 15 cows within each target group (e.g. cows within 2 weeks prepartum or 2 weeks postpartum) and then evaluate the proportion of sampled animals that fall above the suggested analyte concentration thresholds (Ospina et al., 2010c; Oetzel 2004). Ospina et al. (2010c) demonstrated that when herds had more than 15% of the sampled animals with NEFA or BHBA concentrations above the critical thresholds, these herds had slightly higher disease incidence, poorer reproductive performance and lower 305ME milk yield compared to herds with less than 15% of sampled animals above the critical thresholds.

Presently cow-side BHBA tests are available (e.g. Blood: Precision Xtra, Abbott Laboratories, and Milk: Ketotest, Elanco Animal Health) that make

routine evaluation of BHBA simple, fast and low cost. Blood samples must be sent to a commercial laboratory for the analysis of NEFA, Hp and F.CORT. While most animal health laboratories routinely measure NEFA only a handful offer a Hp assay (e.g. Ontario Veterinary College Animal Health Laboratory, Kansas State University Veterinary Diagnostic Laboratory). Presently, there are no commercial laboratories that offer an assay to measure F.CORT concentrations. However, given the strong relationship that biomarkers of inflammation and stress have with milk yield and reproductive performance, further research will be conducted to develop these analytes as potential tools to help identify opportunities for improved transition cow management.

Conclusions

Elevated concentrations of energy related metabolites such as NEFA or BHBA during the weeks before and after calving have been repeatedly shown to be associated with increased disease risk, low milk yield and poor reproductive performance in dairy cattle. New research now shows that elevated concentrations of biomarkers related to stress and inflammation (plasma haptoglobin and fecal cortisol metabolites) during the period around calving are also associated with decreased milk yield in early lactation and longer days to conception.

The identification of high-risk cattle is an important first step in recognizing that there may be opportunities to improve herd management. Analyte testing may be a tool producers can use to identify high-risk cows or herds; this will not only facilitate prompt treatment but also allow for the consideration of pro-active management changes that may improve the health and well-being of transition dairy cattle.

References

Bertoni, G., E. Trevisi, X. Han, M. Bionaz. 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. J. Dairy Sci. 91:3300-310.

Chapinal, N., M. Carson, T. F. Duffield, M. Capel, S. Godden, M. Overton, J. E. P. Santos, and S. J. LeBlanc. 2011. The association of serum metabolites with clinical disease during the transition period. J. Dairy Sci. 94:4897–4903.

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

Cook, C. J., D. J. Mellor, P. J. Harris, J. R. Ingram, and L. R. Mat- thews. 2000. Hands-on and hands-off measurement of stress. Pages 123–146 in The Biology of Animal Stress. G. P. Moberg and J. A. Mench, ed. CABI Publishing, Wallingford, UK.

Duffield, T. F., K. D. Lissemore, B. W. McBride, and K. E. Leslie. 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. J. Dairy Sci. 92:571–580.

Eckersall, P. D. 2000. Recent advances and future prospects for the use of acute phase proteins as markers of disease in animals. Revue. Med. Vet. 151:577-584.

Hirvonen, J., S. Pyörälä, and H. Jousimies-Somer. 1996. Acute phase response in heifers with experimentally induced mastitis. J. Dairy Res. 63:351-360.

Huzzey, J. M., T. F. Duffield, S. J. LeBlanc, D. M. Veira, D. M. Weary, and M. A. G. von Keyserlingk. 2009. Short communication: Hapto- globin as an early indicator of metritis. J. Dairy Sci. 92:621–625.

Huzzey, J. M., D. V. Nydam, R. J. Grant, and T. R. Overton. 2012. Associations of prepartum plasma cortisol, haptoglobin, fecal cortisol metabolites, and nonesterified fatty acids with postpartum health status in Holstein dairy cows. J. Dairy Sci. 94:5878-5889.

Grummer, R. R., D. G. Mashek, A. Hayirli. 2004. Dry matter intake and energy balance in the transition period. Veterinary Clinics of North America Food Animal Practice.20: 447-470

Ingraham, R. H., L. C. Kappel. 1988 Metabolic profile testing. Veterinary Clinics of North America Food Animal Practice. 4:391-411.

Kaneene, J. B., R. Miller, T. H. Herdt, and J. C. Gardiner. 1997. The association of serum nonesterified fatty acids and cholesterol, management and feeding practices with peripartum disease in dairy cows. Prev. Vet. Med. 31:59–72.

Oetzel, G.R. (2004) Monitoring and testing dairy herds for metabolic disease. Veterinary Clinics of North America Food Animal Practice. 20:651-674.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010a. Evaluation of nonesterified fatty acids and beta-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. J. Dairy Sci. 93:546–554.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010b. Associations of elevated nonesterified fatty acids and beta-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. J. Dairy Sci. 93:1596–1603.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010c. Association between the proportion of sampled transition cows with increased nonesterified fatty acids and beta-hydroxybutyrate and disease incidence, pregnancy rate, and milk production at the herd level. J. Dairy Sci. 93:3995-3601.

Palme, R., C. Robia, S. Messmann, J. Hofer, and E. Möstl. 1999. Measurement of faecal cortisol metabolites in ruminants: A non-invasive parameter of adrenocortical function. Wiener Tierärztliche Monatschrift 86:237–241.

Uchida, E., N. Katoh, and K. Takahashi. 1993. Appearance of haptoglobin in serum from cows at parturition. J. Vet. Med. Sci. 55:893-94

Thun, R., E. Eggenberger, K. Zerobin, T. Lüscher, and W. Vetter. 1981. Twenty-four-hour secretory pattern of cortisol in the bull: Evidence of episodic secretion and circadian rhythm. Endocrinology 109:2208–2212.

Walsh, R. B., J. S. Walton, D. F. Kelton, S. J. LeBlanc, K. E. Leslie, and T. F. Duffield. 2007. The effect of subclinical ketosis in early lactation on reproductive performance of postpartum dairy cows. J. Dairy Sci. 90:2788–2796.