Improving the health status of the animal to enhance the quality of milk

Defining blood and milk biomarkers for healthy status

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OMICS technologies for analyzing diseases in livestock species

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Peripartuerient phase: the last 1 to 2 months of gestation and the first few months after delivery

Transition phase: 3 weeks before to 3 weeks after parturition.

These phases are characterized by dramatic changes in metabolism and host defence mechanisms, that are associated with an increased risk of disease.

Transition phase is an extremely critical period for dairy cows because innate and adaptive immune defense mechanisms are weakest.

They are exposed to considerable hormonal changes and sudden onset of milk production, which impairs immune defense mechanisms.

Kehrli et al., 1989; Mallard et al., 1998; Kimura et al., 1999

Petrera et al. (2014) evaluated blood metabolites during transition phase and showed that the largest and most rapid change in blood metabolite levels occurred within:

1 week prior to the 2 weeks after parturition.

The period of transition between late pregnancy and early lactation presents metabolic challenge due to the high-yielding dairy cow

Haematochemical profiles

are important in evaluating the health status of animals during this transition.

Hagawane et al., 2009; Bell, 2000

The Haematochemical profiles could include:

Blood Cells	-	Total White and Red cells
Renal Function	-	Urea nitrogen and Creatinine
Blood Metabolite	-	Non-Esterified Fatty Acids (**NEFA**) Beta-Hydroxybutyrate **(BHBA)** Glucose Albumin Cholestrerol Triglycerides
Macro Minerals	-	Calcium Phosphorous Magnesium

In periparturient animals increasing numbers of total white blood cells are found in the peripheral blood, mainly due to a rise in neutrophil concentration.

Peak values are observed at parturition, but levels decline shortly after and reach basal conditions within 2 weeks postpartum.

Detilleux et al., 1995; Klinkon and Zadnik, 1999; Orrù et al., 2012

High blood concentrations of glucocorticoids have been suggested as an explanation for the increased neutrophil count around parturition.

Burton et al., 1995; Lee and Kehrli, 1998; Kehrli et al., 1999

Experimental injections with synthetic glucocorticoids
to create artificial stress similar to that in the periparturient period
induced marked neutrophilia and down-regulation of L-selectin
(a cell adhesion molecule found on lymphocytes)
on neutrophil membranes.

**The neutrophils became less able
to migrate at the site of inflammation and
the susceptibility to infections increased**

Burton et al., 1995; Nakagawa et al., 1999; Kulberg et al., 2002

Red blood cell count, hemoglobin, hematocrit,
mean corpuscular volume and mean corpuscular hemoglobin
decreased after calving,

whereas total platelet number increased.

Klinkon and Zadnik, 1999; Orrù et al., 2012

Renal function

principally represented by Urea and Creatinine concentrations, is affected by different physiological phases.

Blood Urea Nitrogen is a measure of tissue mobilization during the periparturient period.

Rastani et al., 2006

Creatinine serum level show the higher levels during the late pregnancy and early lactation.
The increase in serum Creatinine levels could be attributed to the development of the foetal musculature.

Piccione et al., 2012; Roubies et al., 2006

The transition from gestation to lactation is a period of great

METABOLIC stress

for dairy cows

high rates of body condition score losses are associated with a severe negative energy balance status indicated by alterations in blood metabolite and hormone profiles.

Wathes et al., 2009; Heck et al., 2009; Rollin et al., 2010

Specifically, high levels of **NEFA** and **BHBA** concentrations are indicative of lipid mobilization and fatty acid oxidation.

Wathes et al., 2009; Sakha et al., 2006

Higher circulating **NEFA** concentrations in cows during the periparturient period have been associated with:

- hepatic lipidosis	*Bobe et al., 2004; Ingvartsen, 2006*
- left displacement of the abomasum	*LeBlanc et al., 2005*
- retained placenta	*Quiroz-Rocha et al., 2009*
- mastitis	*Moyes et al., 2009*

Cows with subclinical **ketosis** during the first 1 to 2 weeks after calving are at greater risk for:

- metritis	*Duffield et al., 2009*
- left displaced abomasum	*LeBlanc et al., 2005*
- incidence and severity of mastitis	*Kremer et al., 1993; Janosi et al., 2003*

Glucose is required by phagocytic cells
for proliferation, survival and differentiation,
has been shown to be the preferred metabolic fuel during inflammation
for activated neutrophils, macrophages and lymphocytes.

Serum glucose concentrations are lowest in cows during early lactation
low availability may limit the immune function and
increase the risk of infection.
Ingvartsen and Moyes, 2012

Higher prepartal glucose was associated with increased
incidence of lameness, mastitis, and milk fever.
Lower prepartal glucose was associated with metritis and dystocia.
Moyes et al., 2013

The standard to define the degree of change in energy mobilization during early lactation in relation to risk of disease is

Energy Balance

primarily based on: dry matter intake, milk yield and milk components.

Ingvartsen (2006) defined

Physiological Imbalance

as cows whose parameters deviate from the normal and have an increased risk of developing production diseases (clinical or subclinical) and reduced production or reproduction.

Moyes et al. (2013) hypothesized that **Physiological Imbalance**, based on several metabolites in blood, will more directly relate to mechanisms associated with the development of several diseases.

In the last years, several Authors identified plasma **NEFA**, **BHBA** and **Glucose** as the major metabolites that relate to the degree of

Physiological Imbalance

during early lactation.

LeBlanc, 2010; Bjerre-Harpoth et al., 2012; Ingvartsen and Moyes, 2012

Moyes et al. (2013) showed

that cows with higher **Physiological Imbalance** prepartum were at a greater risk for developing diseases (mastitis, metritis, retained placenta), milk fever and lameness after calving.

Higher prepartal **NEFA** and **Glucose** were significantly associated with risk of certain diseases after parturition.

The **Physiological Imbalance** index and plasma **NEFA**

were better predictors of disease

compared with **Energy Balance, Glucose** and **BHBA**.

Macro Minerals

All animals require minerals:

Calcium (Ca), Magnesium (Mg) and Phosphorus (P)
for growth, reproduction and lactation.

They serve as:

catalytic components of enzymes or
regulate several mechanism involved just in pregnancy and lactation.

Samardzija et al., 2011; Tanritanir et al., 2009

As we have seen a number of biomarkers are well described in blood, but are currently less well characterized in milk.

Actual milk biomarkers

The Fat / Protein ratio of milk
is a good risk indicator Negative Energy Balance, ketosis and left displaced abomasum, ovarian cysts, lameness and mastitis.

Levels of risk to Negative Energy Balance:

Fat / Protein	> 1.4	
Protein	< 2.9	
Fat	> 4.8	
Lactose	< 4.5	
Urea nitrogen	> 30	mg / 100mL

Levels of risk to mastitis:

Somatic Cell Count	> 200 10^3 x mL

Potential milk biomarkers

free Glucose, Isocitrate, BHBA	ketosis and fatty liver
D-lactate	acidosis and lameness
Lactate dehydrogenase, Immune biomarkers	mastitis
Progesterone, Immune biomarkers	infertility and metritis

MIR spectra:

fat, protein, lactose, casein, BHBA, aceton, lactoferrin, fatty acids, mineral, nitrogen loss, methane emission

Glycan profiles

Milk biomarkers, production and health data collected together will reflect

- production efficiency
- metabolic status
- fertility
- clinical and subclinical diseases
- environmental footprint

Enjalbert et al., 2001; de Roos et al., 2007; Nielsen et al. 2005; Van Haelst et al., 2008; Soyeurt et al., 2007 and 2012