

# Understanding Inflammation and Immunity to Improve Transition Management

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## Take-Home Messages

- Impairments in immune function and a pro-inflammatory state coincide at the start of lactation in many dairy cows, and are associated with greater risk for disease.
- A growing number of feed and pharmaceutical products are offering a variety of means to attempt to enhance immune function in the transition period, and other tools are being tested for limiting inflammation during the transition period.
- Post-calving anti-inflammatory treatments can, in some cases, dramatically increase milk yield over the entire lactation.
- Inherent links between inflammation and immunity raise important questions about whether dairies can “have their cake and eat it, too”, by improving immunity while avoiding inflammatory condition. These questions are still being resolved; however, several studies point to the suggestion that net benefits on health and productivity can be achieved.
- It is likely that some herds may benefit most from anti-inflammatory strategies, while others may benefit most from immune promotion tools. To date, there has been essentially no research on combinations of these strategies.

## Why worry about immunity?

Large-scale analysis of dairy herd records suggests that, around the globe, transition cow problems account for over half of mature animal health problems on a typical dairy farm. There are some factors that are obvious risks to cows immediately after calving, including the potential for latent mastitis cases to re-emerge at the onset of lactation and the tissue trauma from calving. However, there is also a well-documented alteration in immune function during the weeks around calving (Kerhli, 2015). In particular, the function of innate immune cells seems to be consistently impaired. Innate immune cells are those involved in quick recognition and clearance of pathogens, independent of pathogen-specific memory (antibodies).

Why is the immune system of transition cows suppressed? The exact reasons for decreased immune function during the transition period are complex. However, studies with mastectomized cows made it clear that the primary driver is not gestation and calving, but rather lactation and the metabolic changes that come with it (Nonnecke et al., 2003). Numerous large studies have demonstrated that metabolic diseases (e.g. ketosis) put cows at higher risk of contracting clinical infections; likewise, cows with infectious diseases (e.g. metritis) are also at higher risk of subsequent metabolic disorders. The inter-dependent nature of the immune and metabolic systems in the animal are only now

becoming clear, but high blood ketone and non-esterified fatty acid concentrations as well as hypocalcemia are known to limit the responsiveness of immune cells to pathogenic signals. Cows with excessive body condition experience more dramatic drops in immune function at calving, possibly as a consequence of oxidative stress. As a result, nutrition of the transition cow can have a large influence on immunity during this time, even beyond the vitamins and minerals that have received focus in the past.

There is some direct evidence that poor immune responsiveness in the transition period is predictive for incidence of infections during this time. In one study, 5 of 31 cows were identified as poor immune responders 4 weeks before calving. All 5 of these cows developed clinical infections during the first 2 months of lactation, whereas only 3 of the other 26 cows did so (Catalani et al., 2013). It is likely that the high rate of infections in early lactation can be attributed in part to immunosuppression.

### **What does inflammation have to do with transition cows?**

Inflammation is a key component of the immune response to infection or tissue damage. Immune cells that first sense pathogens or signs of traumatized cells release signals that activate pain sensors, promote blood flow to the local tissue, and cause fever, accounting for the traditional signs of inflammation. Additionally, the systemic effects of inflammation include an alteration of liver function, typically called the acute phase response. Most of these responses are beneficial for recruiting innate immune cells to the site of immune activation and for inhibition of bacterial growth, but they come at a cost to the animal. Importantly, inflammation can occur in the absence of a true pathogen challenge and can occur without the traditional signs of focal pain, swelling, and redness. When blood markers of inflammation are elevated in the absence of clinical signs, it is often referred to as sub-acute inflammation.

The presence of an acute phase response in postpartum dairy cows is well-established (Bradford et al., 2015). Although early studies focused on associations between inflammatory markers and diseases such as mastitis and metritis, numerous studies in the past decade have demonstrated that inflammatory and acute-phase mediators are elevated in the days after parturition, even in cows that are apparently healthy. This growing body of evidence suggests that either the processes of parturition and galactopoiesis induce inflammation directly or that infections or endotoxin affect far more fresh cows than is currently recognized. Whatever the explanation, the prevalence of post-calving inflammation raises important questions about the implications for early lactation cows.

Although most transition dairy cows apparently experience a period of inflammation, the magnitude of this inflammatory condition varies greatly between cows. Bertoni et al. (2008) assessed the importance of this variation by measuring a panel of inflammatory markers and separating transition cows into quartiles for degree of inflammation. Cows in the highest quartile had significantly lower milk yields than those in the lowest quartile throughout the first month of lactation, differing by 20% on day 28 of lactation (Bertoni et al., 2008). One metric that has been used in this respect is paraoxanase, a plasma biomarker that is potently suppressed by a variety of inflammatory stimuli. Transition cows with high paraoxanase concentrations, in addition to having lower concentrations of acute phase proteins and reactive oxygen metabolites, produced 4,346 lb more milk (24%) over 305 days than those in the lowest quartile for paraoxanase (Bionaz et al., 2007). Other findings suggest that stronger inflammatory responses in the first week of lactation are associated with decreased

whole-lactation milk yield (Huzzey et al., 2015). Plasma concentrations of haptoglobin (an acute phase protein) greater than 1.1 g/L were associated with a 2,088 lb decrease in 305-day mature equivalent milk yield, and elevated haptoglobin was also associated with a 19% decreased risk of conception. Abnormally high markers of inflammation are associated with poor production, health, and fertility outcomes.

### **Immune promotion tools**

With the growing interest in animal characteristics influencing infection risk, a number of factors have emerged as important for supporting strong immunity. Data currently available suggest that cows have improved transition immune function when: 1) they are not exposed to significant heat stress during the dry period; 2) they calve with a BCS  $\leq 3.5$ ; 3) they are supplemented with antioxidants during the dry period; 4) total serum calcium concentrations are maintained near 9 mg/dL, and 5) blood BHBA and NEFA concentrations stay below 1 mM during the transition. Considering the immune system of the transition cow does not necessarily require a change in recommendations for management during this period, but can provide additional motivation to prevent heat stress, provide sufficient access to feed, manage body condition, support calcium homeostasis, and monitor oxidative balance.

Beyond these best practices for transition cow management, a variety of dietary and pharmaceutical products are being marketed for the explicit purpose of improving immune function. Vaccines have obviously been a very useful tool in promotion of adaptive immunity for decades, and the ongoing development of a vaccine against metritis-causing pathogens may soon bring a new weapon to bear on a frustrating problem (Machado et al., 2014). On the other hand, pharmacological tools for promotion of innate immunity have not been available for livestock until very recently. Granulocyte colony-stimulating factor (GCSF) is a signal used by the immune system which has been adapted into an injectable prophylactic treatment used prior to the period of immunosuppression. The GCSF treatment stimulates the development and maturation of neutrophils, resulting in a fairly dramatic increase in the population of these key innate immune cells in circulation. In conditions favorable to environmental mastitis, the administration of GCSF significantly decreases the incidence of clinical mastitis (Hassfurther et al., 2015).

Dietary agents are also being used as immune stimulants, although the exact modes of action for these feed additives are more elusive. We recently reported that a dietary yeast product enhanced antibody response to vaccination and stimulated greater gut release of IgA, which is able to bind to and carry pathogens out of the gut (Yuan et al., 2015). A large-scale analysis of commercial farm responses (off-on) to a different feed supplement was presented recently, suggesting beneficial effects on farm-recorded mastitis and mortality (Chapman et al., 2016). Such dietary components can likely alter the responsiveness of the immune system by interacting with immune sentinels lining the gut and/or by altering gut epithelium function, but other mechanisms cannot yet be ruled out.

### **Responses to anti-inflammatory treatments**

Motivated by evidence linking early lactation inflammation to decreased health and productivity, we conducted a study with 78 cows assigned to either control or sodium salicylate delivered via drinking water (2 g/L) for the first 7 days of lactation (Farney et al., 2013). Sodium salicylate is a member of the non-steroidal anti-inflammatory drug (NSAID) class, and is the parent compound of

aspirin. At first the results did not look very promising, with no improvement in metabolic health and no increase in early milk yield. However, as lactation progressed, the oldest cohort of cows treated with salicylate (those in parity 3 and greater) responded by producing 21% more milk over the full lactation, and fully 30% more milk fat, than parity-matched controls. On the other hand, primiparous cows treated with salicylate tended to produce less milk, suggesting a potential parity difference in either baseline inflammatory status or response to inflammatory signals.

We subsequently completed a follow-up study to evaluate whether postpartum treatment of multiparous cows could increase whole-lactation productivity of cows on a commercial farm. To facilitate treatment in a commercial setting, we shortened postpartum treatment to 3 days (sodium salicylate) or 1 day (meloxicam) and compared them to placebo treatments (Carpenter et al., 2016a) across 153 cows. Despite this very limited treatment window, cows treated with either NSAID produced about 10% more milk over the whole lactation compared to placebo. Over the 365 days following treatment, meloxicam also tended to delay removal from the herd based on survival analysis ( $P = 0.06$ ; 30, 35, and 38 of 51 cows remained at 365 d postpartum for control, salicylate, and meloxicam, respectively). Meloxicam primarily affected early-lactation culling, and health records recorded by the farm suggested that metabolic disorders accounted for most of this decrease.

Several other groups in a variety of countries have failed to observe significant impacts of postpartum anti-inflammatory treatment on milk yield, and it remains to be seen whether a treatment paradigm can be found that is consistently effective. However, we believe that impacts on long-term milk yield likely require treatment relatively early after calving (though not before the placenta is cleared); that treatment responsiveness is not limited to cows with calving difficulties; and that milk yield must be monitored for at least 60 days into lactation to have a good chance to observe the impact of anti-inflammatory treatment.

The use of anti-inflammatory drugs to treat nonspecific postpartum inflammation is not currently approved. Therefore, it is worthwhile to consider whether some feed ingredients might offer the same anti-inflammatory benefits without the use of regulated pharmaceuticals.

Polyphenols are a diverse class of compounds found in nearly all plants in varying concentrations. Some polyphenols have been clearly shown to have potent anti-inflammatory effects, and a recent study demonstrated some exciting responses in dairy cattle during the transition period. Winkler et al. (2015) reported that cows supplemented with a feed supplement containing green tea and curcuma extract for the close-up period through 9 weeks in milk had decreased plasma NEFA concentrations after calving and produced approximately 10 lb/day more milk in weeks 4 – 8 of lactation.

A different nutritional approach to limiting inflammation is to use omega-3 fatty acids. These polyunsaturated fatty acids have well-described mechanisms underlying their anti-inflammatory effects, although efficiently delivering them to the small intestine is a challenge in ruminants because of ruminal biohydrogenation of dietary unsaturated fatty acids. Nevertheless, feeding whole flaxseed (omega-3 source) compared to sources of omega-6 fatty acids increased plasma glucose and decreased plasma ketones in fresh cows; more surprisingly, the anti-inflammatory omega-3 source resulted in greater phagocytic activity of circulating leukocytes (Gandra et al., 2016). Although this finding of improved metabolic and immune function is exciting, previous studies have reported indications of less responsive immune systems in cows fed omega-3 sources (Lessard et al., 2003;

Silvestre et al., 2011), and such findings are more in line with research in rodents. Perhaps the key to beneficial impacts of omega-3 fatty acids on both inflammation and immunity is an improvement in metabolic profile.

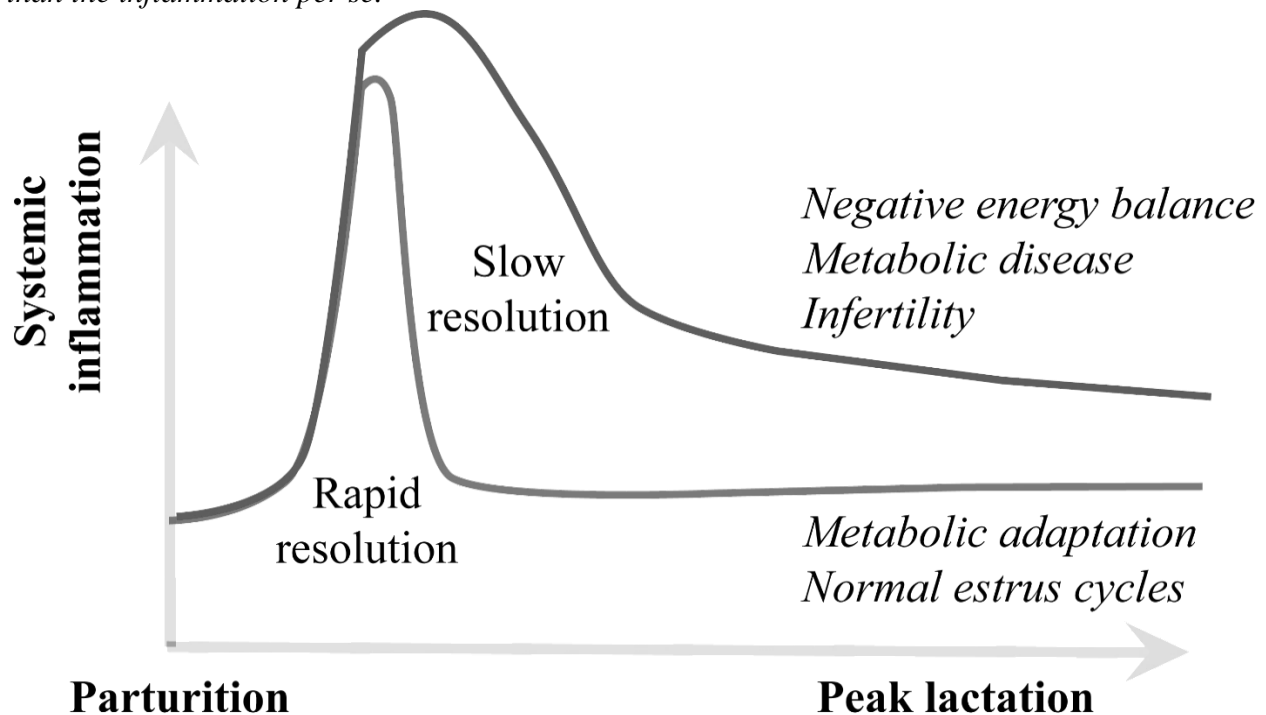
### **Is there an inherent conflict between promoting immunity and preventing excessive inflammation?**

Because inflammation is a core component of the immune system's response to an infection, it is logical to ask whether anti-inflammatory strategies may worsen the immunosuppression that is already recognized as a problem in transition cows. In fact, Nightingale et al. (2015) demonstrated that transition cows with the most dramatic inflammatory profiles also had the most potent measures of neutrophil function. One interpretation of these findings is that transition cows are adapted to respond to immunosuppression with a compensatory inflammatory state.

Inherent conflicts between anti-inflammatory strategies and potent immune responses are also suggested by findings of increased infection rates following NSAID treatments in some small studies and greater mortality rates following pathogen challenges in mice genetically engineered to allow for endogenous omega-3 synthesis (Bradford et al., 2015). Likewise, dietary supplementation of an immune stimulant resulted in an increased acute phase response to endotoxin (Brandão et al., 2016), suggesting that at least some means of enhancing immunity will likely promote inflammation as well. Still, these results do not necessarily mean that a more appropriate balance cannot be achieved. In fact, the immune stimulant described above resulted in increased milk yield (Brandão et al., 2016), and as mentioned before, post-calving meloxicam treatment increased both milk yield and herd retention (Carpenter et al., 2016a).

One question that has not yet been addressed in observational studies is whether the pattern of inflammation impacts long-term outcomes. We hypothesize that brief spikes in inflammatory signals that are resolved in the first 3-4 days of lactation may support immunity and physiological adaptations to lactation. However, failure to rapidly resolve these signals may lead to a variety of adverse impacts that ultimately impair productivity, health, and fertility (Figure 1). We hope that new data will begin to address this question in the coming few years.

**Figure 1.** Hypothetical impacts of brief, rapidly resolved postpartum inflammation versus sustained inflammation. It is proposed that lack of resolution leads to impaired health and productivity rather than the inflammation per se.



### Choosing the right strategy for each herd

In research with anti-inflammatory agents, there have been some marked differences across studies that, while not allowing strong conclusions, hint at predictors for success with these tools. First, treatment with Banamine shortly before and shortly after calving disrupted the normal process of calving and placental expulsion (Newby et al. 2017), resulting in increased incidence of stillbirths (if given before calving) and metritis (if given after calving). This particular approach to combatting calving-associated pain and inflammation is not advised until at least 24 hours after calving.

Second, we have seen variable milk production responses to NSAID treatment even when using identical strategies. Treatment with sodium salicylate for 3 days starting 24 hours after calving increased whole-lactation milk yield by more than 2,000 pounds in one study (Carpenter et al., 2016a), whereas in a follow-up study, we observed no milk response at all (Carpenter et al., 2016b). One potentially relevant difference between the cohorts in these two studies is that the responsive group had substantially greater post-calving inflammation, as the mean plasma haptoglobin concentration was more than twice as high in the responsive group compared to the unresponsive group on days 3-4 of lactation. Although we have been unable to demonstrate that individual cows with higher haptoglobin concentrations are more responsive to NSAID treatment, differences between these two studies seem to point in that direction.

Finally, it stands to reason that farms with very few infectious disease problems are less likely to have obvious benefits from immune stimulation. As a simple example, on-farm evaluation of

responses to the dietary supplement Omnigen AF showed that decreases in somatic cell count after supplementation began were greatest in herds that started with relatively high somatic cells (Chapman et al., 2016).

Although there is little research basis for this suggestion, mechanisms connecting inflammation and immunity lead to the suggestion that cows in different herds may struggle with different mixtures of transition disorders because of imbalances between pro- and anti-inflammatory signals; excessive inflammation in some herds and inadequate immunity in others. Based on this logic, herds that have relatively high prevalence of infectious diseases in early lactation might be wise to focus on trying immune support tools in an attempt to enhance cows' abilities to combat pathogens. Conversely, herds with more metabolic disorders in early lactation should consider implementing anti-inflammatory management and nutritional strategies. Combinations of both types of supplements may or may not have additive benefits - these interactions simply have not been studied.

### **Conclusions**

The growing number of tools available to aid cows successfully transitioning to lactation is exciting, but, as always, the devil is in the details. Several pharmaceutical and feed additive strategies have strong evidence for specific benefits, but individual farms differ in important ways that can lead to unique questions about secondary effects that are less clear. In particular, unresolved questions about tradeoffs between inflammatory status and immunity make it difficult to give one-size-fits-all recommendations when the transition problems encountered on one farm can differ so dramatically from another. Based on evidence available today, farms with more frequent infectious disease problems are encouraged to explore opportunities to promote immune function, whereas those with prevalent metabolic disorders should perhaps focus more on anti-inflammatory strategies. Research on combinations of such strategies are needed before recommendations can be provided with confidence.

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